# CYTO-TAXONOMIC STUDIES IN ORYZOPSIS

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## Introduction

The genus *Oryzopsis*, like other genera of the tribe Agrostideae, is difficult to circumscribe satisfactorily. It is characterized by discontinuities between small groups of closely related species. On some characters it approaches the neighboring genera *Nassella* and *Piptochaetium*, while on others its close affinity to *Stipa* has resulted in an almost arbitrary assignment of certain species to either genus. Three main aggregates of species within *Oryzopsis* have in the past been recognized as different genera or have been combined with *Nassella* and *Piptochaetium* to form a more comprehensive genus. The whole problem of where to draw the generic boundaries in this part of the subtribe Stipeae is still an open one. Whether *Oryzopsis* is a natural genus is a question which will bear much weight in the ultimate disposition of its component species, as well as of the smaller adjacent genera.

The history of *Oryzopsis* bears out the fact that considerable uncertainty exists as to its proper circumscription. The genus was founded by MICHAUX (**12**) in 1803 on the basis of a single species, *Oryzopsis asperifolia* Michx., whose range he cited as Hudson Bay to Quebec. Previous to that date four Old World species from the general region of the Mediterranean now included in *Oryzopsis* had been described and referred to *Agrostis* and *Milium*. In 1812 BEAUVOIS (**3**) established the genus *Piptatherum*, which eventually included these species of *Milium* and *Agrostis* as well as two which were described later. The western American species now known as *Oryzopsis hymenoides* (Roem. and Schult.) Ricker was described as a *Stipa*, but in 1818 NUTTALL (**14**) founded the genus *Eriocoma* on the basis of this single species.

The three genera *Oryzopsis*, *Piptatherum*, and *Eriocoma* were subsequently reduced to the status of sections in the comprehensive genus *Urachne* Trin., which was established in 1820. The genus *Piptochaetium*, which had been founded by PRESL (**15**) in 1830, was next reduced to sectional rank and included in *Urachne*. To these four sections TRINIUS then added the section *Nassella*. The last two sections consist mostly of temperate South American species. Thus the genus *Urachne* included all the present species of *Oryzopsis* which had been described under various genera up to that time. In addition, it included the sections *Piptochaetium* and *Nassella*, which are now excluded from *Oryzopsis*.

BENTHAM (**4**) in 1882 recognized that *Urachne* was a synonym of *Oryzopsis* and adopted the latter name. He retained the sections *Piptatherum* and *Eriocoma* as defined by TRINIUS but included *Piptochaetium* and *Nassella* in his section *Euoryzopsis*. HACKEL (**6**) later excluded *Piptochaetium* and *Nassella* from the section *Euoryzopsis*, giving them generic rank. This left the genus *Oryzopsis* with three sections, *Euoryzopsis*, *Piptatherum*, and *Eriocoma*, an interpretation which has persisted up to the present time.

In contrast to this complicated derivation of the present genus *Oryzopsis* is that of the closely allied genus *Stipa*. Less than 10% of the species of *Stipa* treated by HITCHCOCK (9) have been referred to other genera at any time, while all the species of *Oryzopsis* considered in this study—except the last two to be described—have borne from one to four different generic names. Only four of them were originally described under *Oryzopsis*. In the opinion of HITCHCOCK (8), *Stipa*, *Oryzopsis*, *Nassella*, and *Piptochaetium* form a "well-marked group" in the subtribe Stipeae. The contrast in their treatment by systematists suggests that *Stipa* is a large, coherent swarm of species and that the other genera represent smaller peripheral swarms whose precise natural positions are not clear.

Judging in part from the routine transfers that have been made in the past between *Oryzopsis* and other genera, it is obvious that a continuation of the same practice will not alone clarify the generic entities in this general group. A critical re-examination of morphological characters is desirable in order to evaluate the degree of relationship within and between these aggregates of species.

Discontinuities in general are largely the result of isolation, and changes in the chromosomal complement frequently act as efficient isolating mechanisms. Thus, if the information gained by re-examining morphological characters is supplemented by a knowledge of the nature of the interrelationships as revealed by cytological studies, it should be possible to draw the generic boundaries with greater confidence. With this aim, the present cyto-taxonomic study of *Oryzopsis* is undertaken, giving special attention to its confusing points of contact with *Stipa*.

## Material and methods

Data on morphological characters were obtained from herbarium sheets selected by the use of TIPPETT's (17) random sampling numbers so as to represent in general the geographic ranges of the various species studied. The information presented in tables 1–10 is in most cases based on measurements from two spikelets obtained from each specimen with the aid of TIPPETT's numbers. The illustrations of floral parts, except in the case of *Oryzopsis hymenoides*, were prepared from photographs made at a uniform magnification for all species.

Cytological materials were obtained from plants transplanted directly to the greenhouse from their natural habitat or from nursery plots. In a few cases plants were grown from seed. Root tips were uniformly fixed in Randolph's chromo-acetic-formalin. Camera-lucida drawings were made of root-tip metaphase figures at a magnification of 2420 ×. The number of drawings made varied from twenty-five in some of the species with low chromosome numbers to six in the species with the highest chromosome number. In most cases the number of satisfactory counts far exceeded the number of drawings made. The idiogram for each species shown in figures 24–32 is based on the average of three such drawings. For each drawing, the chromosomes were measured on a millimeter scale and estimated to the nearest tenth of a millimeter, paired on the basis of length and gross morphology, and arranged in order of decreasing length. These chromosome lengths were then averaged for the three drawings. Only the longer chromosome of each pair is illustrated.

## Taxonomic summary of Oryzopsis

Characters of the lemma are in general relied upon to distinguish *Oryzopsis*

from *Stipa*. In *Oryzopsis* the lemma is relatively short, broad, and usually indurate, with a blunt callus and deciduous awn. In *Stipa* the lemma is long and slender, usually less indurate, with a sharp-pointed callus and a persistent awn. Also, the panicle in *Oryzopsis* is usually open, while in *Stipa* it is usually contracted. As is frequently the case, none of these characters is wholly reliable when considered independently. Even when considered together, they do not effect a clear distinction between the two genera, for certain species are either intermediate in the expression of a majority of these characters or else distinctly resemble *Oryzopsis* in some and *Stipa* in others. The tendency on the part of systematists has been to assign the doubtful cases to *Oryzopsis*.

Intergradation of these two genera occurs in North America in the sections *Euoryzopsis* and *Eriocoma*. The Old World species are included in the section *Piptatherum*, and they constitute a clearly defined group which cannot readily be confused with *Stipa*.

## Sections of Oryzopsis

### Section 1.—*Piptatherum* (Beauv.) Benth.

Lemma obovate or elliptic, mostly dorsiventrally compressed and greatly indurate (not indurate in *O. miliacea*), glabrous or short pubescent, pale to dark brown or black, without a distinct callus; awn straight or flexuous and deciduous; palea at maturity broadly exposed between edges of lemma; glumes acuminate or acute, exceeding the lemma, 3–9 nerved, first glume longer than or equal to second; panicle open, branches distant and spreading, spikelet-bearing toward the ends; style branches reflexed at anthesis.

*Oryzopsis miliacea* (L.) Benth. and Hook.
*O. virescens* (Scop.) Beck.
*O. paradoxa* (L.) Nutt.
*O. coerulescens* (Desf.) Hack.
*O. holciformis* (Bieb.) Hack.
*O. racemosa* (J. E. Smith) Ricker

### Section 2.—*Eriocoma* (Nutt.) Benth.

Lemma broadly fusiform, indurate, villous dark brown at maturity, with distinct, oblique callus; awn straight and deciduous; palea at maturity exposed between edges of lemma; glumes acuminate, greatly exceeding the lemma, 3–7 nerved, panicle diffuse with long capillary pedicels or contracted with erect branches and pedicels.

*Oryzopsis hymenoides* (Roem. and Schult.) Ricker

### Section 3.—*Euoryzopsis* Benth.

Lemma obovate or fusiform, not greatly indurate, pubescent and pale or dark brown at maturity (glabrous in *O. hendersoni* and mostly glabrous in *O. micrantha*), with short callus; awn straight to geniculate, deciduous or fairly persistent; palea at maturity narrowly exposed between edges of lemma (or completely inclosed in convolute lemma in *O. asperifolia* and *O. hendersoni*); glumes obtuse or acute, equal to the lemma (acuminate and exceeding the lemma in *O. micrantha*), mostly 1–3 nerved (5–9 nerved in *O. asperifolia* and *O. hendersoni*), second glume longer than or equal to first; panicle narrow or open; style branches erect at anthesis.

*Oryzopsis micrantha* (Trin. and Rupr.) Thurb.
*O. pungens* (Torr.) Hitchc.
*O. canadensis* (Poir.) Torr.
*O. exigua* Thurb.
*O. kingii* (Boland.) Beal
*O. asperifolia* Michx.
*O. hendersoni* Vasey

KEY TO NORTH AMERICAN SPECIES OF ORYZOPSIS

1. Glumes longer than lemma, at least 3-nerved, equal or the first exceeding the second
   2. Lemma glabrous or short pubescent with hairs less than 0.5 mm. long, somewhat dorsiventrally compressed, with indistinct callus.....Section *Piptatherum*
      3. Lemma less than 3 mm. long, glabrous, pale at maturity (introduced) *O. miliacea*
      3. Lemma more than 6 mm. long, short pubescent, black at maturity *O. racemosa*
   2. Lemma pubescent with hairs more than 1 mm. long, fusiform, with distinct callus
      4. Lemma less than 4 mm. long; awn straight, less than 9 mm. long Section *Eriocoma*
         5. Panicle mostly diffuse (rarely contracted); hairs of lemma more than 2 mm. long.....*O. hymenoides*
         5. Panicle contracted with erect branches; hairs of lemma less than 2 mm. long *O. hymenoides* var. *contracta*
      4. Lemma more than 4 mm. long; awn once geniculate, more than 10 mm. long (see JOHNSON, 11) Hybrids, *Stiporyzopsis*
1. First glume shorter than or equal to lemma (longer than lemma in *O. micrantha*), some glumes only 1-nerved in some species, equal, or the second exceeding the first Section *Euoryzopsis*
   6. Lemma 2–2.5 mm. long, glabrous or rarely pubescent.....*O. micrantha*
   6. Lemma more than 3 mm. long
      7. Lemma pubescent, at least at base, pale
         8. Lemma pubescent throughout, 3.5–5 mm. long
            9. Awn less than 2 mm. long *O. pungens*
            9. Awn 5 mm. long or more
               10. Panicle branches spreading *O. canadensis*
               10. Panicle branches erect
                  11. Awn about 5 mm. long, sharply once geniculate *O. exigua*
                  11. Awn about 10 mm. long, curved or weakly geniculate.....*O. kingii*
         8. Lemma densely pubescent on callus, nearly glabrous above, about 6 mm. long.....*O. asperifolia*
      7. Lemma glabrous, brown at maturity *O. hendersoni*

## Natural affinities in Oryzopsis

### I. DIPLOID SPECIES OF SECTION PIPTATHERUM

Five Old World species of *Oryzopsis* included in this study—*O. miliacea* (L.) Benth. and Hook., *O. virescens* (Scop.) Beck., *O. paradoxa* (L.) Nutt., *O. coerulescens* (Desf.) Hack., and *O. holciformis* (Bieb.) Hack.—were placed by TRINIUS and RUPRECHT (18) in the section *Piptatherum* of the genus *Urachne*. They are here referred to the section *Piptatherum* under *Oryzopsis* and are treated as diploids on the basis of chromosome counts for three of them. The two species of unknown chromosome numbers are included because of their obvious close affinity on morphological characters to the other three. These five species range from southern Europe and northern Africa to Asia Minor and northeastward into Asiatic Russia. While they show variation in the size and number of spikelets and in the density of the panicle, they nevertheless are a distinctly homogeneous group whose coherence is traceable through characters of the floral parts, inflorescence, and to some extent in vegetative characters (figs. 1–12; tables 1, 2).

It will be noted (table 1) that while the lemma is greatly variable in size, from a length of about 2 mm. in *O. miliacea* (fig. 1) to about 7 mm. in *O. holciformis* (fig. 10), the species figured here still shows a relationship in its general features. The lemma varies slightly in outline (table 2), from obovate in *O. miliacea* and *O. virescens* to elliptic in *O. paradoxa*, *O. coerulescens*, and *O.*

FIGS. 1–12.—Floral parts: Figs. 1, 2, *Oryzopsis miliacea;* fig. 1, lemma; fig. 2, spikelet. Figs. 3–5, *O. virescens;* fig. 3, lemma side view; fig. 4, lemma front view; fig. 5, spikelet. Figs. 6, 7, *O. paradoxa;* fig. 6, lemma side view; fig. 7, lemma front view. Figs. 8, 9, *O. coerulescens;* fig. 8, lemma side view; fig. 9, lemma front view. Figs. 10–12, *O. holciformis;* fig. 10, lemma side view; fig. 11, lemma front view; fig. 12, spikelet. All ×10.

*holciformis*. It is quite uniform throughout this group in lacking a clearly differentiated callus. In the case of *O. coerulescens* (fig. 8) and *O. holciformis* (fig. 10), the basal end of the floret is reduced rather than modified to form a callus, as in the other sections of the genus. In *O. miliacea* the edges of the lemma form

TABLE 1

MEAN LENGTHS (WITH S.D.) IN MILLIMETERS OF FLORAL AND VEGETATIVE PARTS OF FIVE SPECIES OF ORYZOPSIS OF SECTION PIPTATHERUM

| | O. miliacea | O. virescens | O. paradoxa | O. coerulescens | O. holciformis |
|---|---|---|---|---|---|
| Pedicels: | | | | | |
| Longer | 3.1±0.3 | 15.0±6.5 | 8.9±1.1 | 4.3±1.9 | 10.4±4.9 |
| Shorter | 1.2±0.3 | 10.9±5.3 | 4.4±1.1 | 1.6±1.2 | 5.0±2.5 |
| Glumes: | | | | | |
| First | 3.6±0.4 | 4.6±0.3 | 6.5±0.7 | 7.8±1.1 | 10.2±2.0 |
| Second | 3.2±0.3 | 4.4±0.2 | 5.4±0.7 | 7.1±0.7 | 9.7±1.9 |
| Lemma | 2.2±0.2 | 3.6±0.2 | 4.2±0.3 | 4.3±0.3 | 6.6±1.1 |
| Hairs of lemma | None | 0.3±0.0 | 0.3±0.0 | None | 0.3±0.1 |
| Palea | 1.9±0.2 | 3.1±0.1 | 3.6±0.3 | 3.9±0.3 | 6.0±1.0 |
| Anthers: | | | | | |
| Longest | 1.4±0.1 | 2.3±0.1 | 2.6±0.2 | 2.9±0.3 | 4.0±0.9 |
| Next to longest | 1.4±0.1 | 2.3±0.1 | 2.6±0.2 | 2.9±0.3 | 4.0±0.8 |
| Anther beards | 0.1+0.0 | 0.1±0.0 | None | 0.1±0.0 | Rudimentary |
| Awn | 3.7±0.7 | 12.6±1.1 | 11.7±2.2 | 3.1±1.1 | 10.3±3.2 |
| Ligule | 1.7±0.6 | 0.8±0.3 | 0.7±0.1 | 7.0±2.2 | 8.5±2.1 |
| No. of measurements | 10 | 10 | 4 | 10 | 10 |

TABLE 2

MORPHOLOGICAL CHARACTERS OF FIVE SPECIES OF ORYZOPSIS OF SECTION PIPTATHERUM

| | O. miliacea | O. virescens | O. paradoxa | O. coerulescens | O. holciformis |
|---|---|---|---|---|---|
| Panicle | Open | Open | Open | Open | Open |
| Glumes: | | | | | |
| Shape | Acuminate | Acute | Acuminate | Acuminate | Acuminate |
| No. of nerves— | | | | | |
| First glume | 3 | 3 (5)* | 5 | 5–9 | 5–8 |
| Second glume | 3 | 3 (5) | 3 | 5–7 | 5–7 |
| Lemma: | | | | | |
| Shape | Obovate | Obovate | Elliptic, flat | Elliptic, flat | Elliptic, flat |
| No. of nerves | 3 | 5 (7) | 5 (6) | 5 (6) | 5 |
| Summit | Minutely lobed | Lobed | Short lobed | Minutely lobed | Not lobed |
| Palea: | | | | | |
| Shape | Elliptic | Elliptic | Elliptic | Elliptic | Elliptic |
| No. of nerves | 2 | 2 | 2 (4) | 2 | 2 |
| Anther beards per sac | 3–8 | 0–4 | 0 | 0–5 | 0–7 |
| Awn: | | | | | |
| Persistence | Deciduous | Deciduous | Deciduous | Deciduous | Tardily deciduous |
| Geniculation | Straight | Flexuous | Flexuous | Straight | Flexuous |
| Texture | Scaberulous | Scaberulous | Scaberulous | Scaberulous | Scaberulous |
| Throat of sheath | Glabrous | Sparsely pubescent | Sparsely pubescent | Glabrous | Glabrous |
| Pollen | Normal | Normal | Normal | Normal | Normal |

* Numbers in parentheses represent a few exceptional cases.

two small lobes in front of the awn. In *O. virescens* these lobes are more distinct, but in the other species they are reduced, being absent in *O. holciformis*. Together with this reduction of the callus and the lobes, there is a correlated dorsiventral flattening and induration of the lemma. All these features seem to be further correlated with increase in size. The small, thin lemma of *O. miliacea* also has only three nerves, while the larger, indurate lemmas of the other species considered here have from five to seven nerves. The lemma is typically glabrous or sparsely short pubescent, varying from pale in *O. miliacea* to dark brown in *O. virescens*, *O. paradoxa*, and *O. coerulescens*, and black in *O. holciformis*.

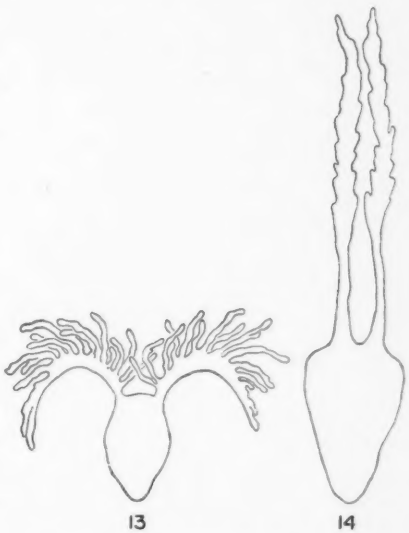
The awn in all these species is weak. In all of them except *O. holciformis* it separates readily at the point of articulation with the lemma. Such a point of articulation is very obscure in *O. holciformis*, and the awn is tardily deciduous, usually breaking off and leaving a jagged edge. *O. coerulescens* has a very much reduced and early deciduous awn. It is shorter than the body of the lemma. This reduction and early deciduousness of the awn occur sporadically in other groups of the Stipeae. Care should therefore be used in attaching any taxonomic significance to it beyond its specific value in these cases.

The anthers of *O. miliacea* are bearded at the apex. In the rest of the species the beards are much fewer. In *O. holciformis* they are rudimentary. None were found in the small amount of material examined of *O. paradoxa*.

A consistent character for the species of this section is found in the style branches (fig. 13), which are reflexed at anthesis, emerging some distance below the summit of the lemma.

In all the Old World species the first glume is longer than the second, and both of them distinctly exceed the lemma (tables 1, 3). They vary from 3-nerved in *O. miliacea* to 8- or 9-nerved in *O. coerulescens* and *O. holciformis*. As compared with other sections, the glumes in this section are somewhat inflated, loosely investing the lemma.

The panicle is open and characterized by distant, spreading branches which bear




FIGS. 13, 14.—Style branches: Fig. 13, *Oryzopsis miliacea*. Fig. 14, *O. micrantha*. ×40.

their spikelets distally. In *O. miliacea* the branches are several at each node. In *O. virescens* they are much fewer, and the branches and pedicels are long and capillary, producing a somewhat diffuse panicle. *O. coerulescens* and *O. holciformis* bear the panicle branches mostly singly or in pairs at the nodes and have shorter pedicels than has *O. virescens*.

The leaves in this section are typically broad and flat, the uppermost ones on the culms frequently being reduced in length. This reduction occurs also in the other

sections of the genus. The culms of *O. miliacea* have several nodes, while in the rest of the species the nodes are three or less per culm, as in other sections of *Oryzopsis*.

TABLE 3

RATIOS OF AVERAGE GLUME LENGTH TO LEMMA LENGTH AND OF LENGTH OF FIRST TO LENGTH OF SECOND GLUME IN ORYZOPSIS

| Species | Av. glume / Lemma | Glume 1 / Glume 2 |
|---|---|---|
| Diploid species: | | |
| Section Piptatherum— | | |
| O. miliacea | 1.55 | 1.12 |
| O. virescens | 1.25 | 1.05 |
| O. paradoxa* | 1.42 | 1.20 |
| O. coerulescens* | 1.73 | 1.09 |
| O. holciformis | 1.51 | 1.05 |
| Section Euoryzopsis— | | |
| O. micrantha | 1.41 | 0.97 |
| O. pungens | 0.88 | 1.03 |
| O. canadensis* | 1.10 | 0.97 |
| O. exigua* | 0.95 | 0.93 |
| O. kingii | 1.09 | 0.85 |
| Polyploid species: | | |
| Section Piptatherum— | | |
| O. racemosa | 1.14 | 1.00 |
| Section Euoryzopsis— | | |
| O. asperifolia | 1.04 | 0.96 |
| O. hendersoni* | 1.06 | 0.98 |

*Chromosome numbers not known.

Of the various species in the section *Piptatherum*, *O. virescens* is most nearly representative of the entire genus *Oryzopsis* on a number of characters. It is not excessively specialized in those characters which are peculiar to the section *Piptatherum*. From the condition found in this species, specialization within the section *Piptatherum* involves some reduction of the callus, greater induration and dorsiventral flattening of the lemma and increase in its size, together with an increase in length and nervation of the glumes. *O. holciformis* is in these respects the most highly specialized species in this section. *O. miliacea* resembles the more highly specialized species in the shape and relative lengths of the glumes, but the small size of its floral parts and the lack of induration of its lemma suggest reduction. Also it departs from sectional characters in type of panicle and number of nodes per culm. Characters approaching those of *O.*

TABLE 4

CHROMOSOME NUMBERS IN SPECIES OF ORYZOPSIS AND STIPA

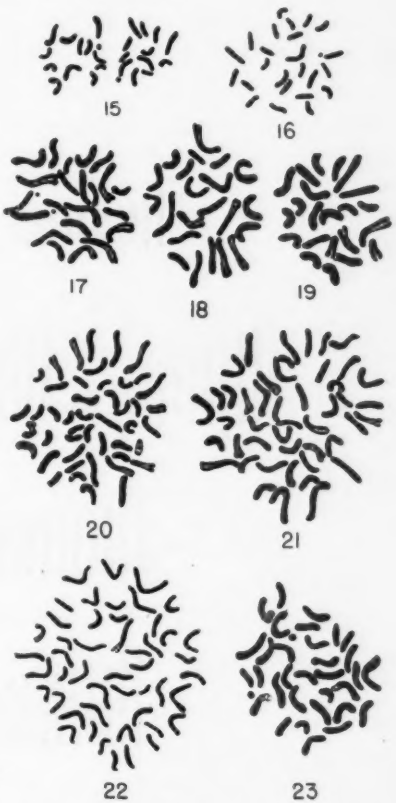
| Species | Number | | Previous record | |
|---|---|---|---|---|
| | n | 2n | 2n | Authority |
| Section Piptatherum: | | | | |
| O. miliacea | 12 | 24 | 24 | Avdulov, 1928 |
| O. virescens | ..... | ..... | 24 | Avdulov, 1928 |
| O. holciformis | ..... | 24 | ..... | ..... |
| O. racemosa | ..... | 46 | ..... | ..... |
| Section Euoryzopsis: | | | | |
| O. micrantha | ..... | 22 | ..... | ..... |
| O. pungens | ..... | 22 | ..... | ..... |
| O. kingii | ..... | 22 | ..... | ..... |
| O. asperifolia | ..... | 46 | ..... | ..... |
| Section Eriocoma: | | | | |
| O. hymenoides | 24 | 48 | ..... | Stebbins and Love, 1941 |
| Genus Stipa: | | | | |
| S. webberi | 16 | 32 | ..... | ..... |
| S. pinetorum | ..... | ..... | 32 | Stebbins, unpublished |
| S. sibirica | 12 | 24 | 24 | Avdulov, 1928 |

*virescens*, which appears to show neither excessive specialization nor reduction, would serve not only as a possible point of divergence for the other species of the section *Piptatherum* but also as a plausible source of characters for the sections *Euoryzopsis* and *Eriocoma*. That the section *Piptatherum* may be ancestral to both of the other sections, at least in part, is indicated by chromosome numbers.

The differentiation of such diverse species as *O. miliacea* and *O. holciformis* in the section *Piptatherum* has been accomplished with scarcely any detectable alteration in the karyotype. AVDULOV (1) reports somatic complements of 24 chromosomes for *O. miliacea* and *O. virescens*. The count for *O. miliacea* has been verified in this study (table 4). In addition, original counts for *O. holciformis* made in this study show that it also has 2n = 24 chromosomes (table 4). The metaphase figures of *O. miliacea* and *O. holciformis* (figs. 15, 16) show no great differences. The chromosomes, as compared with other species of *Oryzopsis*, are small, both narrower and shorter. In each case two chromosomes of about average length bear satellites. In the particular figure of *O. holciformis* shown in figure 16 only one satellite was visible.

Idiograms (figs. 24, 25) of a chromosome set of each species based on three somatic metaphase figures show the chromosomes of both species to vary in length from almost 2$\mu$ to less than 1$\mu$. The satellite is associated with a chromosome of approximately the same length in each case. It is doubtful whether much significance can be attached to minor variations within the set as revealed by these idiograms. In *O. holciformis*, however, the first or longest chromosome is a third again as long as the second.

The count of 2n = 24 chromosomes for *O. holciformis* obtained in this study adds further evidence to that already cited by AVDULOV (2) to the effect that the basic haploid number in *Oryzopsis* is 12. AVDULOV (1) also reports a count of 2n = 24 for *Stipa sibirica* Lam. and concludes (2) that 12 is the basic haploid number for that genus as well. His count for *S. sibirica* is verified in this study by



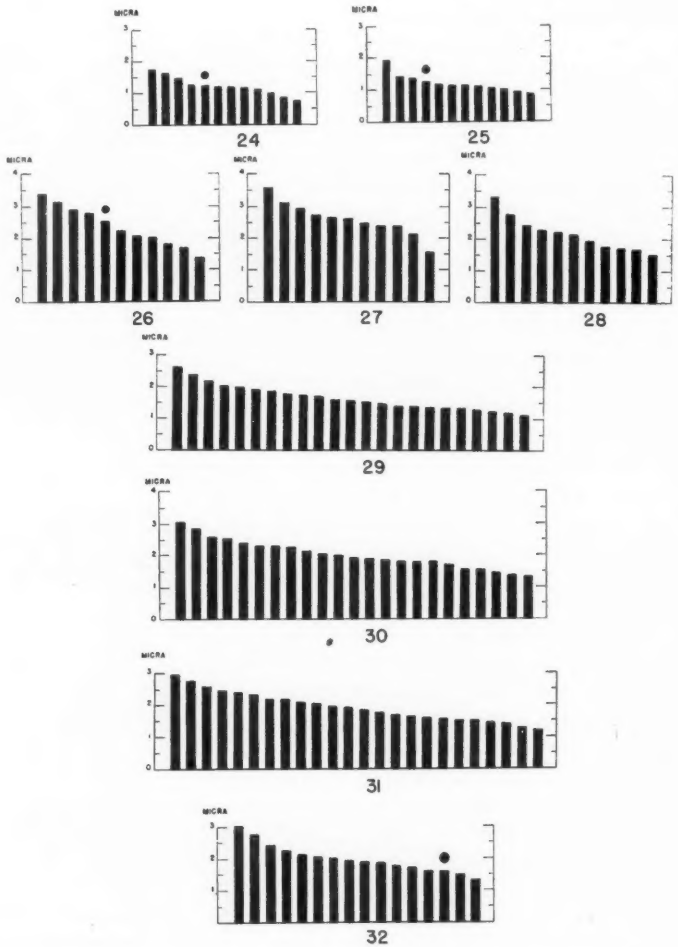

FIGS. 15–23.—Metaphase chromosomes from root tips: Fig. 15, *Oryzopsis miliacea*, 2n = 24. Fig. 16, *O. holciformis*, 2n = 24. Fig. 17, *O. micrantha*, 2n = 22. Fig. 18, *O. pungens*, 2n = 22. Fig. 19, *O. kingii*, 2n = 22. Fig. 20, *O. racemosa*, 2n = 46. Fig. 21, *O. asperifolia*, 2n = 46. Fig. 22, *O. hymenoides*, 2n = 48. Fig. 23, *Stipa webberi*, 2n = 32. All × 2420. (Fig. 22 redrawn from JOHNSON and ROGLER, Amer. Jour. Bot.)

several counts of n = 12 obtained at diakinesis.

The species of the section *Piptatherum* considered here are treated as diploids on the basis of x = 12, although evidence to be discussed later indicates that the primary basic number for both *Stipa* and *Oryzopsis* possibly is x = 6, as suggested by STEBBINS and LOVE (16).

The following herbarium specimens[1]

[1] The herbaria from which specimens were loaned are designated by letters as follows: University of California, Berkeley (UC); United States National Museum (US); Missouri Botanical Garden (MBG); University of Idaho, Southern Branch, Pocatello




FIGS. 24–32.—Idiograms of chromosomes at somatic metaphase: Fig. 24, *Oryzopsis miliacea*. Fig. 25, *O. holciformis*. Fig. 26, *O. micrantha*. Fig. 27, *O. pungens*. Fig. 28, *O. kingii*. Fig. 29, *O. racemosa*. Fig. 30, *O. asperifolia*. Fig. 31, *O. hymenoides*. Fig. 32, *Stipa webberi*.

were used in compiling the data for tables 1 and 2:

*Oryzopsis miliacea.*—France: Bellevue, 1902, Bertrand (UM); Annot, 1902, Legrelle (UM). Canary Islands: Cook 739 (MBG). Palestine: Jerusalem, Dinsmore and Meyers 2146a (UM). Tunisia: Gabes, Pitard 288 (MBG).

*Oryzopsis virescens.*—Rumania: "Ad thermas Herkulis," Thaisz 15 (UM); 1923, Borza and Nyarady (MBG); between Plavisevita and Dubova, 1910, Peterfi (MBG). Hungary: Buda (MBG 210644). Yugoslavia: Gottschee, 1826, Müller (MBG).

*Oryzopsis paradoxa.*—Algeria: Tlemcen, 1856 (MBG 210637); 1906–07, Gandoger (MBG).

*Oryzopsis coerulescens.*—Greece: Macedonia (MBG 210619). Turkey: Smyrna, 1827, Fleischer (MBG). Sicily: Pisano (MBG 210617). "Rupibus Montis Catharinae" (probably Mt. Sinai): 1835, Schimper (MBG). France: Aude, La Nouvelle, 1901, Sennen (UM).

*Oryzopsis holciformis.*—Rumania: Orsova, Degen 3991 (MBG); 1910, Peterfi (MBG); between Plavisevita and Dubova, Degen 16 (UM). Palestine: Jerusalem, Meyers and Dinsmore 2687 (UM). Persia: Shiraz, 1845, Hohenacker (MBG).

## II. Diploid species of section Euoryzopsis

Seven North American species of *Oryzopsis* are included in the section *Euoryzopsis*. Five of these—*O. micrantha* (Trin. and Rupr.) Thurb., *O. pungens* (Torr.) Hitchc., *O. canadensis* (Poir.) Torr., *O. exigua* Thurb., and *O. kingii* (Boland.) Beal—form a basal group in that section and are here treated as diploids, on the basis of chromosome counts for three of them and on the strength of morphological similarity on the part of the other two species. Trinius and Ruprecht (18) included *O. micrantha* in their section *Oryzopsis*, which was about equivalent to the present section *Euoryzopsis*, at the same time pointing out its close affinity to *O. miliacea*—which they placed in the section *Piptatherum*. *O. pungens* (Torr.) Hitchc., which was originally described under the generic name of *Milium*, was included by Trinius and Ruprecht in their section *Oryzopsis* as a synonym of *O. canadensis* (Poir.) Torr. Hitchcock (7) recognized them as distinct species. *O. canadensis* and *O. kingii* were originally described under *Stipa*. *O. exigua* is the only one of these five species originally described under its present generic name. While this group of species is coherent on a number of characters, *O. micrantha* is somewhat detached from the rest by its affinity to the section *Piptatherum*.

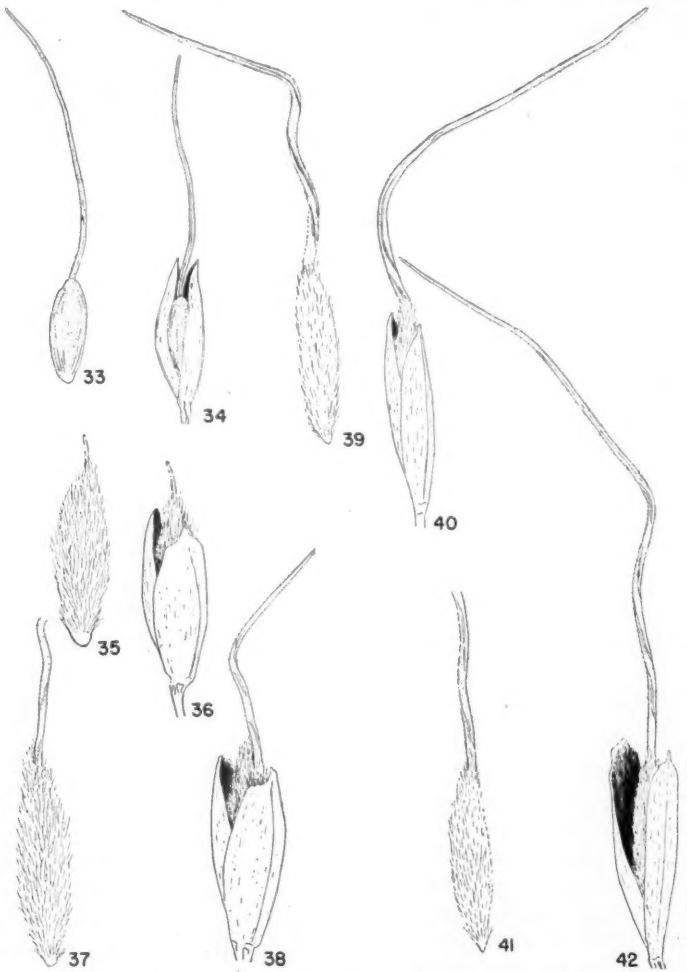
In contrast to the section *Piptatherum*, this group of species (figs. 33–42; tables 5, 6) shows no induration nor dorsiventral flattening of the lemma, nor any appreciable variation in its size. The lemma in *O. micrantha* (fig. 33) is obovate and mostly glabrous, although a less common form has a pubescent lemma. In the other four species it is pubescent and fusiform, attaining a narrow, stipoid form in *O. kingii* (fig. 41). In this group, departure from the characters of the genus as a whole is expressed in greater differentiation of a callus and the development of a strong, twisted awn, which in some species is geniculate and persistent. These are the features which in their more extreme expression constitute the generic characters of *Stipa*. In fact, on the basis of morphological characters there is no definite line of

(UIS); University of Minnesota (UM); State College of Washington, Pullman (WS); University of Wyoming (UWy); University of Colorado (UCo).

demarcation between *Stipa* and this section of *Oryzopsis*.

In *O. micrantha* the callus is weakly developed, as in the section *Piptatherum*. In *O. pungens* (fig. 35) it is blunt and slightly swollen, while in *O. exigua* (fig. 37), *O. canadensis* (fig. 39), and *O. kingii* it is longer and more clearly differentiated from the body of the lemma. In *O. kingii* it is as sharp as in some species of *Stipa*.

The awn in *O. micrantha* is weak and flexuous, as in the section *Piptatherum*. In *O. pungens* it is greatly reduced, but




FIGS. 33–42.—Floral parts: Figs. 33, 34, *Oryzopsis micrantha;* fig. 33, lemma; fig. 34, spikelet. Figs. 35, 36, *O. pungens;* fig. 35, lemma; fig. 36, spikelet. Figs. 37, 38, *O. exigua;* fig. 37, lemma; fig. 38, spikelet. Figs. 39, 40, *O. canadensis;* fig. 39, lemma; fig. 40, spikelet. Figs. 41, 42, *O. kingii;* fig. 41, lemma; fig. 42, spikelet. All ×10.

such an extreme expression of this character apparently has no significance beyond the species in which it occurs, as previously pointed out. In *O. exigua* the awn is about 7 mm. long, sharply once geniculate, and twisted. In *O. canadensis* it is longer, twisted, and obscurely twice geniculate. In *O. kingii* the awn reaches

TABLE 5

MEAN LENGTHS (WITH S.D.) IN MILLIMETERS OF FLORAL AND VEGETATIVE PARTS OF FIVE SPECIES OF ORYZOPSIS OF SECTION EUORYZOPSIS

| | O. micrantha | O. pungens | O. canadensis | O. exigua | O. kingii |
|---|---|---|---|---|---|
| Pedicels: | | | | | |
| Longer | 3.1±0.6 | 5.8±1.3 | 7.7±1.9 | 7.0±1.7 | 4.4±0.8 |
| Shorter | 1.4±0.4 | 3.0±0.9 | 4.3±2.0 | 2.4±1.3 | 1.8±0.6 |
| Glumes: | | | | | |
| First | 3.2±0.3 | 3.3±0.5 | 3.8±0.2 | 4.2±0.5 | 3.5±0.5 |
| Second | 3.3±0.3 | 3.2±0.2 | 3.9±0.1 | 4.5±0.5 | 4.1±0.5 |
| Lemma | 2.3±0.2 | 3.7±0.2 | 3.5±0.3* | 4.6±0.4 | 3.5±0.3 |
| Hairs of lemma | None | 0.4±0.0 | 0.5±0.0* | 0.4±0.1 | 0.4±0.0 |
| Palea | 2.0±0.2 | 3.4±0.2 | 2.9±0.3* | 4.3±0.5 | 2.7±0.2 |
| Anthers: | | | | | |
| Longest | 1.1±0.1 | 2.1±0.2 | 2.0±0.1* | 2.4±0.3† | 1.7±0.2 |
| Next to longest | 1.1±0.1 | 2.1±0.2 | 2.0±0.1* | 2.4±0.3† | 1.7±0.1 |
| Anther beards | None | Rudimentary | 0.1±0.0 | 0.5±0.2 | 0.1±0.7 |
| Awn | 6.5±1.4 | 1.2±0.2 | 8.5±0.8 | 5.6±1.1 | 11.1±1.7 |
| Ligule | 1.7±0.3 | 1.8±0.5 | 1.9±0.5 | 3.0±0.7 | 2.0±0.4 |
| No. of measurements | 22 | 16 | 8 | 16 | 16 |

* Based on 6 measurements.
† Based on 13 measurements.

TABLE 6

MORPHOLOGICAL CHARACTERS OF FIVE SPECIES OF ORYZOPSIS OF SECTION EUORYZOPSIS

| | O. micrantha | O. pungens | O. canadensis | O. exigua | O. kingii |
|---|---|---|---|---|---|
| Panicle | Open | Mostly narrow | Open | Narrow | Narrow |
| Glumes: | | | | | |
| Shape | Acuminate | Obtuse or acute | Obtuse or acute | Obtuse or acute | Obtuse |
| No. of nerves— | | | | | |
| First glume | 1–3 (5)* | 1–3 (5) | 1–3 | 1–3 (5) | 0–1 (3) |
| Second glume | 1–3 (5) | 1–3 (5) | 1–3 (5) | 1–3 (5) | 1 (3) |
| Lemma: | | | | | |
| Shape | Obovate | Fusiform | Fusiform | Fusiform | Fusiform |
| No. of nerves | 5 | 5 (7) | 5 | 5 (7) | 3 (5) |
| Summit | Not lobed | Minutely lobed | Not lobed | Lobed | Minutely lobed |
| Palea: | | | | | |
| Shape | Broad elliptic | Elliptic | Elliptic | Elliptic | Elliptic |
| No. of nerves | 2 | 2 | 2 | 2 (4) | 2 |
| Anther beards per sac | 0–1 (2) | 0 (2) | 0–4 | 0–3 (5) | 3–7 |
| Awn: | | | | | |
| Persistence | Deciduous | Deciduous | Tardily deciduous | Tardily deciduous | Mostly persistent |
| Geniculation | Flexuous | Straight | Twice (weakly) | Once | Curved |
| Texture | Scaberulous | Scaberulous | Scaberulous | Scaberulous | Scaberulous |
| Throat of sheath | Glabrous | Glabrous | Glabrous | Glabrous | Glabrous |
| Pollen | Normal | Normal | Normal | Normal | Normal |

* Numbers in parentheses represent a few exceptional cases.

its greatest development and is twisted and weakly geniculate. In *O. micrantha* and *O. pungens* the awn is early deciduous. In *O. exigua* and *O. canadensis* it is deciduous at maturity, but in *O. kingii* it is often quite persistent, as in the genus *Stipa*. Thus the characters which are usually relied upon to separate the two genera break down within this group of stipoid species of *Oryzopsis*.

In the section *Euoryzopsis* the second glume is longer than the first, except in *O. pungens* (tables 3, 5). In *O. micrantha* (fig. 34; table 6) the glumes are acuminate and exceed the lemma. In this respect they resemble the section *Piptatherum*. However, they are thin and frequently only 1-nerved, suggesting the reduction of the glumes—which is more clearly expressed in the other four species of the section *Euoryzopsis* considered here. These species (figs. 36, 38, 40, 42; tables 5, 6) show reduction in nervation and length of glumes to the condition found in *O. kingii*, which has obtuse, mostly 1-nerved glumes that are shorter than the lemma.

The panicle in *O. micrantha* is open, often with reflexed branches similar to that of the section *Piptatherum*. In *O. pungens* it is mostly narrow but sometimes open, as it is also in *O. canadensis*. In *O. exigua* and *O. kingii* it is narrow, as in the genus *Stipa*. The anthers of these species are mostly beardless, or have only a few small beards, as in the section *Piptatherum*. *O. kingii* has three to seven beards per anther sac. The leaves in all these species are narrow and in most of them involute, in contrast to the wide, flat leaves of the section *Piptatherum*.

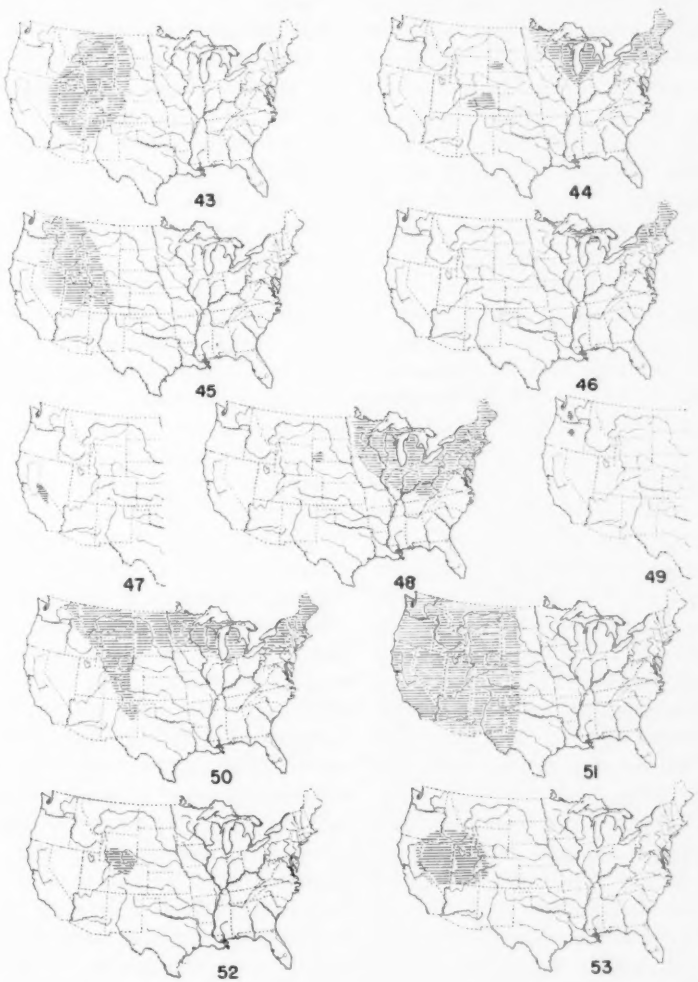
Another feature which appears effectively to separate these two groups is the style branches, which in the section *Euoryzopsis* (fig. 14) are erect at anthesis, emerging at the summit of the lemma, while in the section *Piptatherum* (fig. 13) they are reflexed, emerging below the summit.

*O. pungens* and *O. canadensis* are close together taxonomically, differing mostly in the character of the awn. They also occupy similar ranges (figs. 44, 46), with *O. pungens* more widely distributed in the United States. Authentic material of *O. canadensis* is scarce. Only four collections, ranging from northern Labrador to the New England states and Michigan, have been examined in this study. The range of *O. pungens* extends westward in United States and Canada, probably coming in contact with the range of *O. exigua* (fig. 45). On the other hand, *O. kingii* (fig. 47) is endemic to the meadows at high altitudes in the central Sierra Nevada Mountains, where it occurs abundantly. The geographic range of *O. micrantha* (fig. 43) is roughly similar to that of *O. exigua*.

Thus, while this group of species is coherent on a number of characters, it is apparent that at the more specialized extreme *O. kingii* intergrades with the genus *Stipa*, while at the less specialized extreme *O. micrantha* shows an admixture of characters of *Piptatherum*. *O. kingii*, which was transferred from *Stipa*, is so closely allied to that genus on most characters that its assignment to either *Oryzopsis* or *Stipa* is largely arbitrary. Its narrow, non-indurate lemma, with a sharp callus and strong, twisted, persistent awn, its narrow panicle, and slender, involute leaves are characters which would place this species in *Stipa* were it not for the gradation toward *Oryzopsis* supplied by the other stipoid species. The affinity of *O. micrantha* to the section *Piptatherum* is recognized by ELIAS (5), who placed it in that section. The admixture of *Piptatherum* characters in *O. micrantha* is evidenced by its flexuous

awn and weak callus, its open panicle with reflexed branches, and its acuminate glumes. In these features *O. micrantha* is abruptly set off from the other four stipoid species in the section *Euoryzopsis* considered here.

Aside from these characters, similarity between *O. miliacea* and *O. micrantha* appears to be mostly superficial. The lemmas of both are of about the same length and smaller than in any other species of their respective sections. This apparent




FIGS. 43–53.—Geographic distribution of North American species of *Oryzopsis* and *Stipa webberi:* Fig. 43, *O. micrantha*. Fig. 44, *O. pungens*. Fig. 45, *O. exigua*. Fig. 46, *O. canadensis*. Fig. 47, *O. kingii*. Fig. 48, *O. racemosa*. Fig. 49, *O. hendersoni*. Fig. 50, *O. asperifolia*. Fig. 51, *O. hymenoides*. Fig. 52, *O. hymenoides* var. *contracta*. Fig. 53, *Stipa webberi*.

reduction has left the glumes of *O. micrantha* longer than the lemma, simulating the condition in *O. miliacea*, but other features of the glumes in the former species still show its sectional affinity. The second glume exceeds the first, and both show the reduction in nervation characteristic of the section *Euoryzopsis*. The major affinity of *O. micrantha* to that section is further evidenced by its narrow, involute leaves, its erect style branches, and its geographic range. To this may be added the evidence from chromosome numbers. Thus, by virtue of the combination of characters in *O. micrantha*, the other four stipoid species in the section *Euoryzopsis* articulate with the section *Piptatherum*.

*O. micrantha* was found to have a somatic complement of 22 chromosomes (table 4). As in the Old World species, many figures (fig. 17) were observed in which two chromosomes of about average length bore satellites. The chromosomes, however, are much larger than in either *O. miliacea* or *holciformis*. From the idiogram (fig. 26) it will be noted that they range in length from about 3.5 to 1.5$\mu$, most of them being longer than the longest chromosomes in the Old World species. *O. pungens* and *O. kingii* were also found to have $2n = 22$ chromosomes (figs. 18, 19), with a similar range in length (figs. 27, 28). Cytological material was not obtained for *O. exigua* and *O. canadensis*.

On the basis of karyotype, therefore, *O. micrantha*, *O. pungens*, and *O. kingii* are quite similar, and, except for the combination of characters in *O. micrantha*, the sections *Piptatherum* and *Euoryzopsis*, as judged from the species thus far considered, appear to have diverged along separate lines of specialization, having basic chromosome numbers of $x = 12$ (or 6) and $x = 11$, respectively. Within the section *Piptatherum*, as previously pointed out, specialization involves reduction of the callus, induration and dorsiventral flattening of the lemma and increase in its size, together with an increase in length and nervation of the glumes. Within the section *Euoryzopsis*, *O. micrantha* presents some *Piptatherum* characters, but the rest of this line with $x = 11$ chromosomes illustrates specialization of the awn and callus and the development of a stipoid lemma, together with reduction in length and nervation of the glumes. This line evidently terminates in the genus *Stipa*, where it is represented by several polyploid species.

AVDULOV (**2**) reports counts of $2n = 44$ chromosomes in seven Old World species of *Stipa* and concludes that, in addition to the primary basic number of $x = 12$, a secondary basic number of $x = 11$ has arisen in that genus. NIELSEN (**13**), working with American species, lists $2n = 44$ chromosomes for *Stipa columbiana* Macoun and $2n = 66$ for *S. pulchra* Hitchc. Further evidence for the existence of two basic numbers in *Stipa* is supplied by STEBBINS and LOVE (**16**), who report aneuploidy in that genus, with chromosome counts which suggest a modified polyploid series on the basis of $x = 6$ and $x = 11$. It seems probable that the line tentatively isolated by the change to $x = 11$ chromosomes has evolved through less specialized forms, such as the stipoid species in the section *Euoryzopsis*, and finally has become highly specialized in tetraploid and hexaploid species of *Stipa*. The $x = 12$ (or 6) line, as previously mentioned, is represented in *Stipa* by *S. sibirica* with $2n = 24$ chromosomes, and —as pointed out by STEBBINS and LOVE (**16**)—probably also by a number of American species which have $2n = 36$

chromosomes. The two basic lines in that genus, however, become obscured by the conspicuous aneuploid series. This series may be in part the result of allopolyploidy between the two lines.

The following herbarium specimens were used in compiling the data for tables 5 and 6:

*Oryzopsis micrantha.*—Alberta (Assiniboia): Medicine Hat, Macoun 7505 (UM). North Dakota: Morton County, Colebank 77 (UM). South Dakota: Harney Peak, 1924, McIntosh (UWy). Montana: Lower Sand Coulee, Williams 815 (UM). Nebraska: War Bonnet Canyon, 1890, Williams (UM). Wyoming: New Castle, Pammel 147 (UM). Utah: Fish Lake, Jones 5746 (UWy). Colorado: Larimer County, Gooding 1920 (UCo). Nevada: Deer Creek, Clokey 7825 (UM). New Mexico: Lincoln County, Earle and Earle 165 (UWy). Arizona: Flagstaff, MacDougal 296 (UWy).

*Oryzopsis pungens.*—Quebec: Calumet, 1891, Macoun (UM). New Hampshire: Carroll County, Weatherby and Smith 821 (UM). Vermont: Barnet, 1886, Blanchard (UM). Michigan: Keweenaw County, Hermann 7585 (UM). Wisconsin: Chippewa Falls, Rosendahl and Butters 3148 (UM). Minnesota: Lake County, Lakela 2404 (UM); Itasca Park, Nielsen 1935 (UM). British Columbia: Emerald Lake, Shaw 15 (UM).

*Oryzopsis canadensis.*—Labrador: Ungava River, Spreadborough 13345 (US). Maine: Aroostook County, St. John and Nichols 2123 (US). New Hampshire: Echo Lake, 1878 Faxon (US). Michigan: Marquette County, Hermann 7714 (US).

*Oryzopsis exigua.*—British Columbia: Coldwater River, Copley 56 (WS). Washington: Kititas County, Vasey 34 (WS). Oregon: Wallowa Mountains, Cusick 3302a (WS). Idaho: Custer County, McBride and Payson 3430 (UWy), Nelson and McBride 1555 (WS). Montana: Yellowstone Falls, 1899 Blankenship (UWy). Wyoming: Lincoln County, Payson and Armstrong 3613 (UWy); Uinta County, Nelson and Nelson 6511 (UWy).

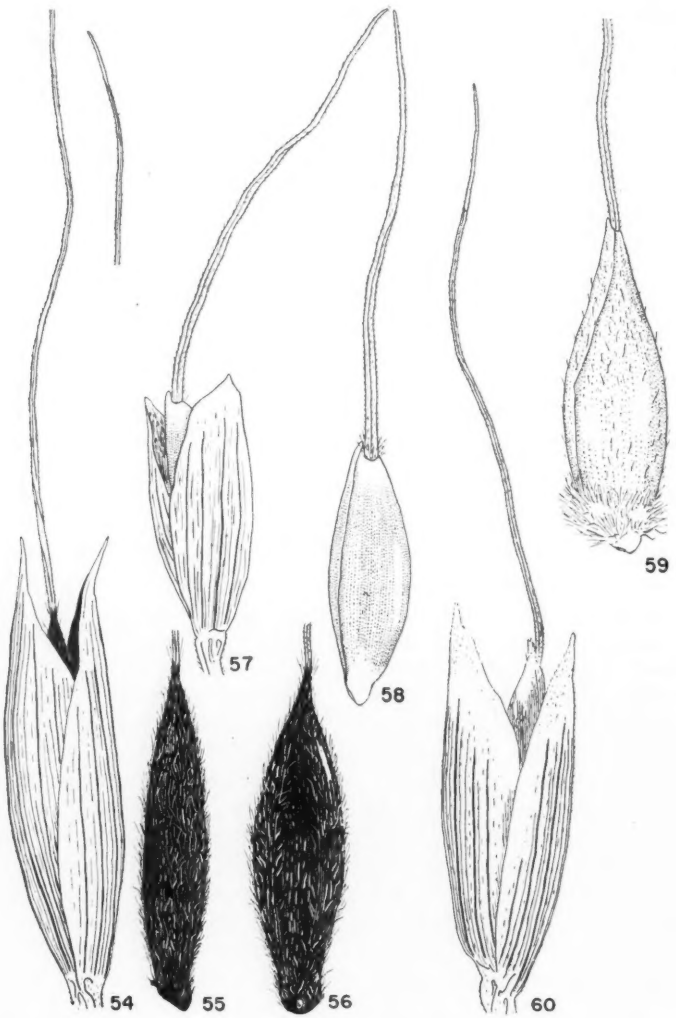
*Oryzopsis kingii.*—California: Mt. Silliman, 1905, Brandegee (UC); Fresno County, Hall and Chandler 601 (UM), Sharsmith 3082 (UC); Tuolumne County, Sharsmith 228 (UC), 1936, Hawbecker (UC); Mono County, Yates 6306 (UC); Mariposa County, 1898, Congdon (UM), 1894, Congdon (UM).

## III. Polyploid species of sections Piptatherum and Euoryzopsis

Three North American species of *Oryzopsis* characterized by large spikelets appear to combine some of the characters of the two lines represented by basic haploid chromosome numbers of $x = 11$ and $x = 12$ (or 6). These are *Oryzopsis racemosa* (J. E. Smith) Ricker, *O. asperifolia* Michx., and *O. hendersoni* Vasey. Chromosome counts were not obtainable for *O. hendersoni*. It is treated together with the other two species because it shows a similar combination of morphological characters.

In *O. racemosa* (figs. 54–56; tables 7, 8) the lemma is very similar to that in *O. holciformis*, being compressed dorsiventrally, greatly indurate, and black at maturity. As in that species also, the point of articulation of the awn at the summit of the lemma is obscure, and the awn is tardily deciduous—often breaking off and leaving an irregular edge. The awn is weak and flexuous, and the panicle is distinctly of the type described for the Old World species belonging to the section *Piptatherum*. The glumes are large and many-nerved, and the leaves are broad. However, the lemma in *O.*

*racemosa* is contracted near the base, forming an indistinct callus. A large scar is present at the point of union with the pedicel. These features suggest the section *Euoryzopsis*, in which the callus is well developed. The lemma is more pubescent than is common in the section *Piptatherum*, and the glumes (table 3) are more nearly equal, more abruptly acuminate, and do not exceed the lemma as much as they commonly do in that section. These are characters which also




FIGS. 54–60.—Floral parts: Figs. 54–56, *Oryzopsis racemosa;* fig. 54, spikelet; fig. 55, lemma side view; fig. 56, lemma front view. Figs. 57, 58, *O. hendersoni;* fig. 57, spikelet; fig. 58, lemma. Figs. 59, 60, *O. asperifolia;* fig. 59, lemma; fig. 60, spikelet. All ×10.

suggest the section *Euoryzopsis*. This species was included in the section *Oryzopsis* by TRINIUS and RUPRECHT (**18**), but it is here included in the section *Piptatherum*—to which it seems more closely related on morphological char-

TABLE 7

MEAN LENGTHS (WITH S.D.) IN MILLIMETERS OF FLORAL AND VEGETATIVE PARTS OF THREE SPECIES OF ORYZOPSIS OF SECTIONS PIPTATHERUM AND EUORYZOPSIS

| | O. racemosa | O. asperifolia | O. hendersoni |
|---|---|---|---|
| Pedicels: | | | |
| Longer | 12.8±2.6 | 10.6±2.7 | 9.0±1.9 |
| Shorter | 5.1±2.0 | 5.6±1.6 | 4.3±1.7 |
| Glumes: | | | |
| First | 8.0±0.5 | 6.6±0.7 | 4.5±0.3 |
| Second | 8.0±0.5 | 6.9±0.7 | 4.6±0.3 |
| Lemma | 7.0±0.4 | 6.5±0.6 | 4.3* |
| Hairs of lemma | 0.4±0.1 | 0.3±0.1 | None |
| Palea | 6.3±0.4 | 5.7±0.4 | 3.0* |
| Anthers: | | | |
| Longest | 4.0±0.4 | 3.7±0.4 | ..... |
| Next to longest | 4.0±0.4 | 3.7±0.4 | ..... |
| Anther beards | None | 0.1±0.0 | 0.2* |
| Awn | 18.3±1.7 | 10.1±1.6 | 9.0±0.5 |
| Ligule | 0.4±0.2 | 0.2±0.1 | 0.5* |
| No. of measurements | 24 | 24 | 5 |

* Based on single measurement.

TABLE 8

MORPHOLOGICAL CHARACTERS OF THREE SPECIES OF ORYZOPSIS OF SECTIONS PIPTATHERUM AND EUORYZOPSIS

| | O. racemosa | O. asperifolia | O. hendersoni |
|---|---|---|---|
| Panicle | Open | Mostly narrow | Narrow |
| Glumes: | | | |
| Shape | Abruptly acuminate | Abruptly acute | Abruptly acute |
| No. of nerves— | | | |
| First glume | 7–9 | 7–9 (11) | 5–7 |
| Second glume | (5) 7–9* | 7–9 | 3–5 |
| Lemma: | | | |
| Shape | Elliptic, flat | Broad fusiform | Broad fusiform |
| No. of nerves | 5 | 7–9 (12) | 5 |
| Summit | Not lobed | Short lobed | Lobed |
| Palea: | | | |
| Shape | Elliptic | Elliptic | Elliptic |
| No. of nerves | 2 or 4 | 2 (4) | 2 |
| Anther beards per sac | 0 | 20–40 | 3–5 |
| Awn: | | | |
| Persistence | Tardily deciduous | Tardily deciduous | Deciduous |
| Geniculation | Flexuous | Curved | Curved |
| Texture | Scaberulous | Scaberulous | Puberulent |
| Throat of sheath | Puberulent | Glabrous | Glabrous |
| Pollen | Normal | Normal | ..... |

* Numbers in parentheses represent a few exceptional cases.

acters. ELIAS (5) also included it in the latter section.

*Oryzopsis racemosa* was found to have 2n = 46 chromosomes (fig. 20). That number would be expected in an allotetraploid between a species with 2n = 24 and a species with 2n = 22 chromosomes. *O. racemosa* may represent such a tetraploid between the lines described for the sections *Piptatherum* and *Euoryzopsis*. The idiogram for this species (fig. 29) shows the chromosomes to range in length from about 2.6$\mu$ to about 1$\mu$. This range takes in almost the entire range of *O. holciformis* plus much of the range of *O. micrantha*, for example. Thus, on the basis of chromosome number and length, together with features of gross morphology, *O. racemosa* seems to represent an allotetraploid between the two lines in *Oryzopsis* with basic numbers of x = 12 (or 6) and x = 11 (fig. 81). It is not presumed that any precise species of *Oryzopsis* still in existence were involved in this synthesis. On morphological characters, *O. racemosa* approaches most closely to *O. holciformis* on the *Piptatherum* side, but in present-day flora their geographic ranges are thoroughly isolated. The geographic range of *O. racemosa* (fig. 48) includes that of *O. pungens* and *O. canadensis*, at least in the United States.

*O. asperifolia* (figs. 59, 60; tables 7, 8) is the type of the section *Euoryzopsis*. On the majority of characters it is similar to the other species of that section, especially to *O. pungens*. It has a fusiform lemma with a swollen, blunt, pubescent callus. The glumes (table 3) are about equal to the lemma in length, and the second exceeds the first. The panicle is narrow, as is mostly the case in *O. pungens*. These two species are also similar in having the upper culm blades reduced to rudiments sometimes less than 5 mm. long. The basal leaves in both species are erect, firm, scabrous, and sharp-pointed. The geographic range of *O. asperifolia* (fig. 50) includes that of *O. pungens*, and their flowering season is the same—very early spring. These two species are, however, very different in size of plant as well as in size of floral parts, *O. asperifolia* being much the larger. In a few characters *O. asperifolia* shows affinity to the section *Piptatherum*. The lemma is more sparsely pubescent and more indurate than in the smaller species of the section *Euoryzopsis*. The awn is weak and flexuous, and the glumes are wide and many-nerved. These features are present in the section *Piptatherum*. The convolute, lobed lemma of *O. asperifolia* is found in only one other species, *O. hendersoni*.

*O. asperifolia* was also found to have 2n = 46 chromosomes (fig. 21), and may—like *O. racemosa*—represent an allotetraploid between the lines described for the sections *Piptatherum* and *Euoryzopsis* with species having 2n = 24 and 2n = 22 chromosomes, respectively. The idiogram (fig. 30) of *O. asperifolia* shows the chromosomes to range in length roughly from 3 to 1.5$\mu$. That range includes only about three of the longest chromosomes in the idiograms of the diploid species illustrated from the section *Piptatherum* but includes most of the range in chromosome length of the diploid species illustrated from the section *Euoryzopsis*. There is no existing species in the section *Piptatherum* with 2n = 24 chromosomes whose geographic range is in contact with that of *O. asperifolia*, nor is it probable that the establishment of such an allotetraploid is of recent occurrence. The presence of *Piptatherum*-like species in the Tertiary flora of Colorado is evidenced by the work of ELIAS (5), who describes the fossil species

*Paleoeriocoma hitchcocki* from the Middle Pliocene and points out that it resembles the living species of the related section *Piptatherum* of the genus *Oryzopsis* in the dorsiventral flattening of the hull.

*Oryzopsis hendersoni* (figs. 57, 58; tables 7, 8) has been reported from only two localities, Mount Clements, Washington, and the Ochoco National Forest, Oregon (fig. 49). In size of plant and floral parts it is smaller than *O. asperifolia* or *O. racemosa*, but like these it seems to combine some of the characters of the more specialized 24- and 22-chromosome species in the sections *Piptatherum* and *Euoryzopsis*, respectively. The lemma is glabrous and dark brown, as in some species of the former section, but it is convolute as in *O. asperifolia* and has a moderately differentiated callus and a fairly strong awn, as in other species of the section *Euoryzopsis*. The wide several-nerved glumes suggest the section *Piptatherum*, but their length and the contracted panicle suggest the section *Euoryzopsis*. No cytological material was obtained for this species. It is included in the section *Euoryzopsis*.

The following herbarium specimens were used in compiling the data for tables 7 and 8:

*Oryzopsis racemosa*.—Maine: Auburn, 1898, Merrill (UM). Massachusetts: Woburn, 1890, Swan (UM). New York: Canandaigua Lake, 1892, Durand (UM). New Jersey: Sussex County, McKenzie 2308 (UM). Virginia: Giles County, Fogg 14914 (UM). Michigan: Hubbardston (without date), Wheeler (UM). Wisconsin: Devils Lake, 1896, Umbach (UM). Iowa: Ames, 1896, Ball (UM); 1897, Ball (UM). Minnesota: Becker County, Grant 3085 (UM); Hennepin County, 1889, Aiton (UM). South Dakota: Bigstone City, Moore 543 (UM).

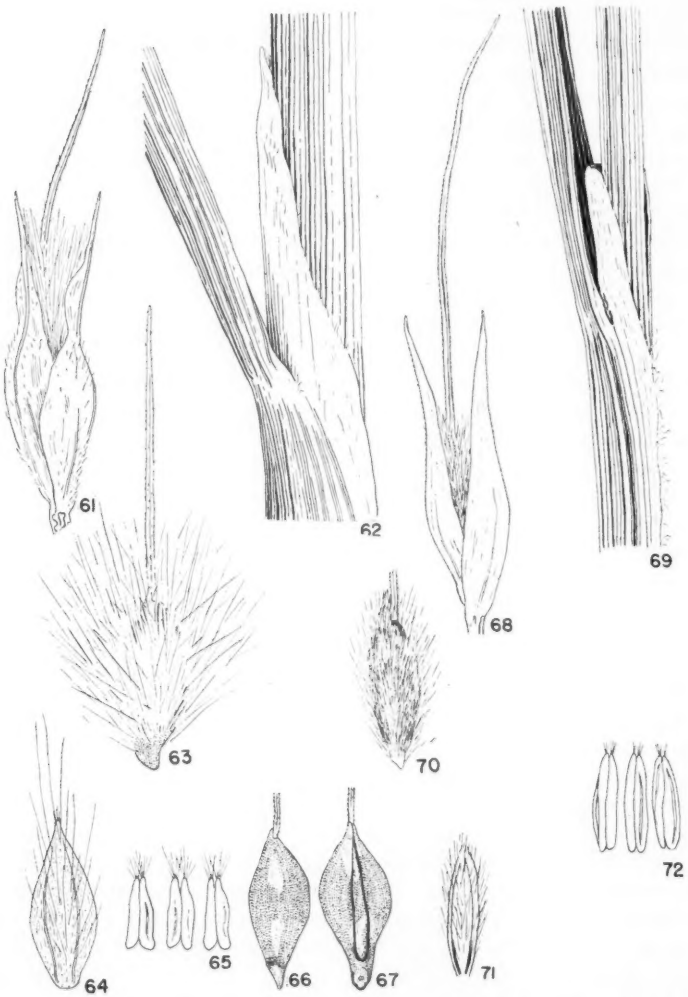
*Oryzopsis asperifolia*.—Ontario: Edmonton, 1893, White (UM). Vermont: Peacham, 1888, Blanchard (UM). New York: Canandaigua, 1892, Durand (UM). Pennsylvania: Potter County, 1926, Sowden (UM). Michigan: Jackson County, 1897, Camp (UM). Wisconsin: St. Croix Falls, Benner 264 (UM). Minnesota: Carlton County, Lakela 1924 (UM). North Dakota: Rolette County, Lunell 873 (UM). South Dakota: Piedmont, 1895, Pratt (UM). Wyoming: Hulett County, Ownbey 598 (UWy). Washington: Metaline Falls, Hitchcock 2936 (WS). Colorado: Larimer County, 1897, Osterhout (UM).

*Oryzopsis hendersoni*.—Washington: Yakima County, Clements Mountain, Henderson 2249 Isotype (WS).

## IV. Section Eriocoma

*Oryzopsis hymenoides* (Roem. and Schult.) Ricker (figs. 61-67; tables 9, 10), which belongs to the section *Eriocoma*, is characterized by a greatly indurate, broadly fusiform lemma (figs. 63, 66, 67) with a distinct, oblique callus and lobes at the summit. In its induration and somewhat in its outline it is similar to the lemma of *O. virescens* (figs. 3, 4), which belongs to the section *Piptatherum*, but the callus in *O. hymenoides* distinguishes it clearly. The lemma is clothed with long silky hairs almost equal in length to the lemma itself, and has a straight, early deciduous awn only slightly longer than the lemma. It has five nerves, with exceptional cases of three. The anthers are conspicuously bearded at the apex. The glumes are long acuminate and invest the lemma closely, the first being commonly 3-nerved and the second 5-nerved. The panicle is diffuse, with divaricate, long, capillary pedicels which are twice as long as the awned floret. These features also suggest the panicle of *O. virescens*.

Thus, in several features *O. hymenoides* shows affinity to the section *Piptatherum*, but it differs from the species of that section in the presence of a callus, in the long hairs of the lemma, and in the glumes which closely invest the lemma. There is little if any resemblance to the section *Euoryzopsis* in these features, except in the callus. The glumes are even more narrowly acuminate than those in *Piptatherum* and differ greatly from the blunt glumes in the section *Euoryzopsis*.




FIGS. 61–72.—Floral and vegetative parts: Figs. 61–67, *Oryzopsis hymenoides;* fig. 61, spikelet; fig. 62, ligule and throat of sheath; fig. 63, lemma; fig. 64, palea; fig. 65, anther set; fig. 66, lemma side view with hairs removed; fig. 67, same, front view. Figs. 68–72, *O. hymenoides* var. *contracta;* fig. 68, spikelet; fig. 69, ligule and throat of sheath; fig. 70, lemma; fig. 71, palea; fig. 72, anther set. All ×10.

TABLE 9

MEAN LENGTHS (WITH S.D.) IN MILLIMETERS OF FLORAL AND VEGETATIVE PARTS OF ONE SPECIES AND A VARIETY OF ORYZOPSIS OF SECTION ERIOCOMA AND OF TWO SPECIES OF STIPA

| | O. hymenoides* | O. hymenoides var. contracta | S. webberi | S. pinetorum |
|---|---|---|---|---|
| Pedicels: | | | | |
| Longer | 14.7±4.4 | 7.8±2.2 | 8.4±2.5 | 6.0±0.9 |
| Shorter | 11.0±3.8 | 3.4±1.4 | 4.0±1.8 | 2.7±0.6 |
| Glumes: | | | | |
| First | 6.3±1.6 | 5.8±0.4 | 8.5±0.7 | 7.9±0.5 |
| Second | 5.6±0.8 | 5.9±0.3 | 8.5±0.7 | 7.8±0.5 |
| Lemma | 3.3±0.3 | 3.2±0.2 | 5.5±0.5 | 4.5±0.1 |
| Hairs of lemma | 2.9±0.4 | 1.3±0.1 | 2.6±0.3 | 3.2±0.3 |
| Palea | 2.8±0.3 | 2.9±0.2 | 4.7±0.3 | 2.5±0.1 |
| Anthers: | | | | |
| Longest | 1.3±0.3 | 1.8±0.1‡ | 2.4±0.5 | 2.6±0.4 |
| Next to longest | 1.2±0.2 | 1.8±0.1‡ | 2.4±0.5 | 2.6±0.3 |
| Anther beards | 0.4±0.1 | 0.3±0.0‡ | None | Occasional |
| Awn | 4.5±1.1 | 7.4±0.7 | 6.3±0.9 | 12.4±0.8 |
| Ligule | 4.7±1.0† | 4.5±1.0§ | 0.3±0.1 | 0.2±0.0 |
| No. of measurements | 60 | 8 | 12 | 5 |

* Data for O. hymenoides from JOHNSON and ROGLER, Amer. Jour. Bot.

† Based on 43 measurements. ‡ Based on 4 measurements. § Based on 15 measurements.

TABLE 10

MORPHOLOGICAL CHARACTERS OF ONE SPECIES AND A VARIETY OF ORYZOPSIS OF SECTION ERIOCOMA AND OF TWO SPECIES OF STIPA

| | O. hymenoides* | O. hymenoides var. contracta | S. webberi | S. pinetorum |
|---|---|---|---|---|
| Panicle | Diffuse | Contracted | Narrow | Narrow |
| Glumes: | | | | |
| Shape | Ovate | Ovate | Lanceolate | Lanceolate |
| No. of nerves— | | | | |
| First glume | 3 (5)† | 3 (+2 short) | 3 (5) | 3 |
| Second glume | (3) 5 (7) | 3 (5) | 3 (5) | 3 |
| Lemma: | | | | |
| Shape | Broad fusiform | Broad fusiform | Narrow fusiform | Narrow fusiform |
| No. of nerves | (3) 5 | 3 (5) | 5 | 5 |
| Summit | Lobed | Long lobed | Long lobed | Long lobed |
| Palea: | | | | |
| Shape | Elliptic | Elliptic | Narrow elliptic | Narrow elliptic |
| No. of nerves | 2 | 2 | 2 | 2 |
| Anthers: | | | | |
| Dimorphism | Equal | Equal | Equal | Equal |
| Abortion | All normal | All normal | All normal | All normal |
| No. of beards per sac | (4) 10–12 (18) | 0–6 | 0 | 0–(4) |
| Awn: | | | | |
| Persistence | Deciduous | Deciduous | Deciduous | Persistent |
| Geniculation | Straight | Straight | Weakly bent | Twice geniculate |
| Texture | Scaberulous | Scaberulous | Scaberulous | Scaberulous |
| Throat of sheath | Pubescent | Glabrous | Glabrous | Glabrous |
| Pollen | Normal | Normal | Normal | Normal |

* Data for O. hymenoides from JOHNSON and ROGLER, Amer. Jour. Bot.

† Numbers in parentheses represent a few exceptional cases.

The hairs of the lemma also are much longer than in either of those sections. These characters, however, are found in the genus *Stipa*, in which the majority of species have long, narrowly acuminate glumes and some species have pilose lemmas.

The somatic chromosome number of *O. hymenoides*, as reported by STEBBINS and LOVE (16) and confirmed by JOHNSON and ROGLER (10), is 48. The idiogram (fig. 31) is in general similar to that for the other polyploid species of *Oryzopsis*, showing a range in chromosome length of 3–1.2 μ. On the basis of chromosome number, *O. hymenoides* might be a polyploid from the line in the section *Piptatherum*, with x = 12 (or 6) chromosomes. However, in length fully half the chromosomes of *O. hymenoides* exceed the longest chromosomes of either *O. miliacea* or *O. holciformis*.

It was shown by JOHNSON and ROGLER (10) that *O. caduca* Beal is a sterile hybrid between *O. hymenoides* and *Stipa viridula* Trin. Later, JOHNSON (11) pointed out that *O. bloomeri* (Boland.) Ricker is a parallel sterile hybrid between *O. hymenoides* and *Stipa occidentalis* Thurb., and that a number of species of *Stipa* are involved in producing such hybrids with *O. hymenoides*. The ease with which it hybridizes with various species of *Stipa*, taken together with evidence from morphological and cytological characters, brings out the possibility that *O. hymenoides* is an allopolyploid between the line in *Oryzopsis* with a basic number of x = 12 (or 6) chromosomes and the line in *Stipa* with a similar basic number. Another possibility is that *O. hymenoides* may be derived from the x = 12 (or 6) line of *Stipa* alone without involving any species of *Piptatherum* affinities. This species ranges (fig. 51) throughout the western half of the United States, where the genus *Stipa* is best developed in North America.

A form of *O. hymenoides* with contracted panicles but similar to the species as generally known in other respects has been collected at Dubois, Idaho. Arthur Cronquist 681 (UIS); 1940, Ray J. Davis (UIS).

A previously undescribed variety of *O. hymenoides* (figs. 68–72; tables 9, 10) is represented by four collections from Colorado and Wyoming (fig. 52).

*Oryzopsis hymenoides* (Roem. and Schult.) Ricker var. **contracta**, var. nov., a forma typica differt panicula contracta, ramis et pedicellis erectis, pedicellis brevioribus spiculis (cum aristis), villis lemmatis dimidio brevioribus lemmate, arista 2.5-plo longiore quam lemmate.

Culms erect, tufted, 2.5-5 dm. high; culm leaves 3, smooth, narrowly involute, the basal one about 20 cm. long, the upper one 4–10 cm. long; uppermost sheaths dilated; ligule 3–6 mm. long; panicle 6–20 cm. long, narrowly contracted, the branches slender, erect in pairs, the ultimate pedicels erect, shorter than the awned spikelet; glumes about 5.5–6 mm. long, puberulent to glabrous, narrow-ovate, 3–5-nerved, abruptly acuminate; lemma fusiform, turgid, about 3 mm. long, with lobes extending more than 0.5 mm. beyond the joint of the awn, 3- (rarely 5-) nerved, dark brown at maturity, densely pubescent with hairs less than 1.5 mm. long; awn slightly bent, 7–8 mm. long, scaberulous, deciduous; anthers bearded.

Wyoming: Freezeout Hills, Carbon County, Elias Nelson 4850 (type) (UWy); Laramie, Albany County, Elias Nelson 411 (UWy); Leucite Hills, Sweetwater County, Merrill and Wilcox 84 (UWy). Colorado: Laramie River, Elias Nelson 467 (UWy).

The above cited herbarium specimens were used in compiling the data for *O. hymenoides* var. *contracta* in tables 9 and 10. The data for *O. hymenoides* were taken from JOHNSON and ROGLER (10).

## V. SPECIES EXCLUDED FROM ORYZOPSIS

1. *Oryzopsis caduca* Beal, Bot. Gaz. 15:111. 1890 = × *Stiporyzopsis caduca* (Beal) Johnson and Rogler (*Oryzopsis hymenoides* × *Stipa viridula*). Amer. Jour. Bot. 30:49–56. 1943.

2. *Oryzopsis bloomeri* (Boland.) Ricker Ex Piper, Contrib. U.S. Natl. Herb. 11:109, 1906; based on *Stipa bloomeri* Boland., Proc. Calif. Acad. Sci. 4:168. 1872, shown by Johnson (11) to be a hybrid between *Oryzopsis hymenoides* and *Stipa occidentalis*.

3. *Oryzopsis webberi* (Thurb.) Benth.; Vasey, Grasses U.S. 23. 1883, based on *Eriocoma webberi* Thurb., in S. Wats., Bot. Calif. 2:283, 1880. Sierra Valley, Calif., Boland. = *Stipa webberi* (Thurb.) comb. nov.

In describing this species, THURBER (in WATSON, 19) "doubtfully referred" it to the genus *Eriocoma*, although Bolander, the collector, had sent it to him with a set of his species of *Stipa* "to which he supposed it to belong." There can be little doubt that Bolander was correct. Its assignment to *Eriocoma*, which was known almost exclusively from the single species now known as *Oryzopsis hymenoides*, was apparently done on the basis of a single character. *Stipa webberi* (figs. 77–80; tables 9, 10) resembles *O. hymenoides* in the long hairs of the lemma and in the deciduousness of the awn, but in the majority of characters it differs from *O. hymenoides* and resembles *Stipa pinetorum* Jones (figs. 73–76; tables 9, 10). It has a long, narrow, non-indurate lemma with a slender callus and long, pointed lobes at the summit. This lemma is quite different from that of *O. hymenoides* but very similar to that of *S. pinetorum*. The latter species also bears long hairs on the lemma. The glumes of *S. webberi* are narrow, as in *S. pinetorum*. The anthers are long and slender, of the *Stipa* type and without beards. The panicle in both species is narrow with appressed branches and frequently has the basal portion included within the somewhat inflated uppermost sheath. This is totally unlike the typical panicle in *O. hymenoides* and in detail does not resemble the more narrow panicle of *O. hymenoides* var. *contracta*. The ligule in *S. webberi* is extremely short, less than 0.5 mm. long (as in *S. pinetorum*), while in *O. hymenoides* it is about 5.0 mm. long.

*S. pinetorum* and *S. webberi* (fig. 53) occupy the same geographic range, with the former occurring at high altitudes in open pine woods and the latter on the deserts and plains.

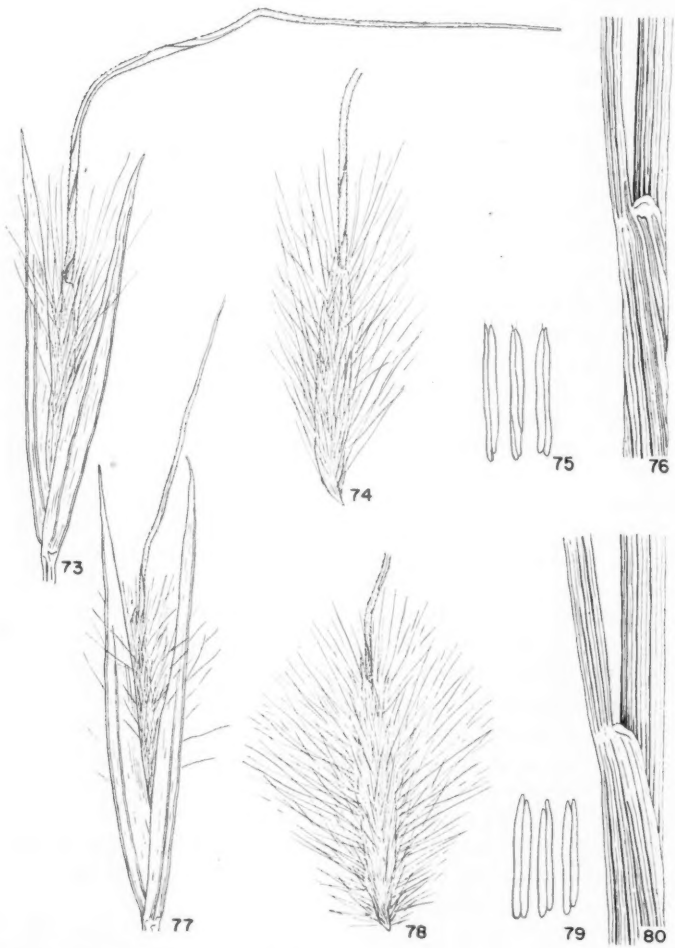
In this study, *S. webberi* was found to have 2n = 32 chromosomes (fig. 23). Several counts of n = 16 were also obtained at diakinesis. This number is not easily explained on the basis of other counts in the genus *Oryzopsis*, where the species seem to be either diploids or polyploids of the euploid type. In *Stipa* an aneuploid series is known to exist. In personal correspondence, STEBBINS has reported a count of 2n = 32 chromosomes for *S. pinetorum* also. The idiogram for *S. webberi* (fig. 32) shows chromosomes corresponding in length to those of the stipoid species and the polyploids of *Oryzopsis*.

It is clear that the only character which would retain this species in the genus *Oryzopsis* is the deciduousness of the awn. Its affinity is obviously with *Stipa*.

The following herbarium specimens were used in compiling the data for tables 9 and 10:

*Stipa webberi.*—Idaho: Owyhee County, reproduced at Pullman, Washington, 1942, Schwendiman (UM). Colorado: Grand Junction, 1900, Stokes (UC). Nevada: Washoe County, Saarni 104 (UC); Elko County, Holmgren 617 (UWy); Esmeralda County, Duran 3088 (UM). California: Lassen County, Keck and Clausen 3749 (UC).

*Stipa pinetorum.*—California: Mono County, Sharsmith 4206 (UM).




FIGS. 73–80.—Floral and vegetative parts: Figs. 73–76, *Stipa pinetorum;* fig. 73, spikelet; fig. 74, lemma; fig. 75, anther set; fig. 76, throat of sheath. Figs. 77–80, *Stipa webberi;* fig. 77, spikelet; fig. 78, lemma; fig. 79, anther set; fig. 80, throat of sheath. All ×10.

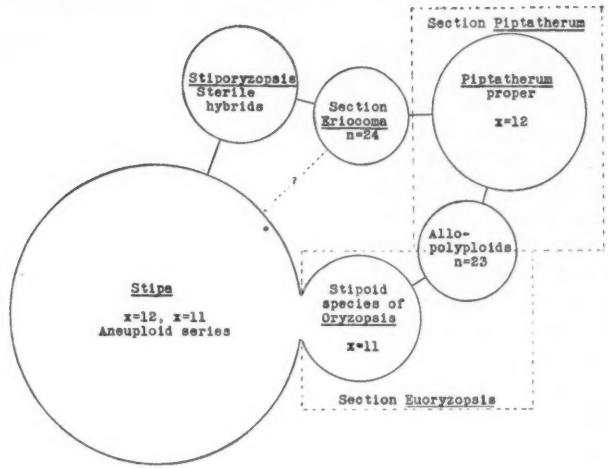
## Conclusions

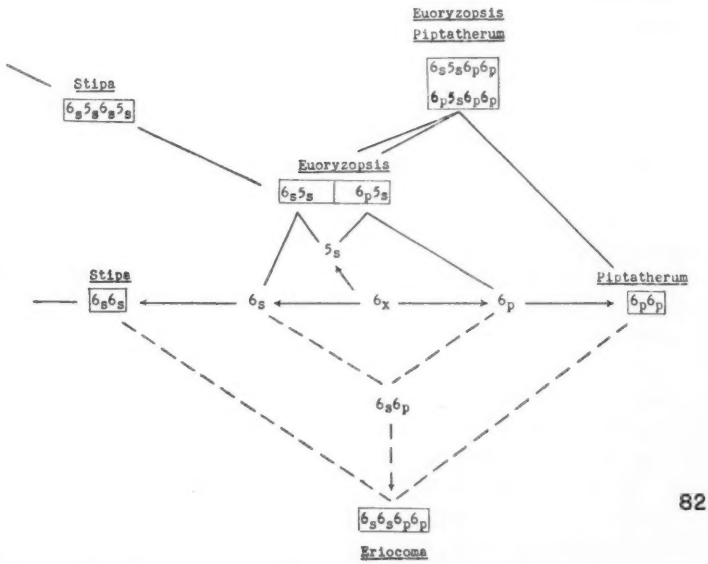
In the subtribe Stipeae the genera *Oryzopsis* and *Stipa* have apparently originated from a common basic stock with x = 6 chromosomes. From this basic stock two diverging lines (fig. 81), each with x = 12 (or 6) chromosomes, seem to have given rise mainly to the genus *Stipa* on the one hand and mainly to the section *Piptatherum* of the genus *Oryzopsis* on the other hand. The absence of a series of intergrading forms indicates that isolation of these two lines is at present fairly complete. However, the polyploid species *O. hymenoides* which constitutes the section *Eriocoma* has the same basic chromosome number and combines some of the more specialized characters from both lines. This suggests that isolation of these lines has been overcome by allopolyploidy in this case.

An early change to a secondary basic number of x = 11 appears to have tentatively isolated a portion of the x = 12 (or 6) line leading to *Stipa*. Some of the less specialized species in this new x = 11 line constitute the major part of the section *Euoryzopsis* in the genus *Oryzopsis*. On morphological characters these species intergrade completely with *Stipa;* but at present, on the basis of x = 11, only diploids are known for *Oryzopsis* and only polyploids for *Stipa*. While the x = 11 line is presumably isolated from the x = 12 (or 6) line in the section *Piptatherum*, recombination between them evidently has occurred through the formation of allopolyploids such as *O. racemosa* and *O. asperifolia*, which combine the chromosome numbers as well as some of the more specialized morphological characters from both lines. Similar recombination between the x = 11 and the x = 12 (or 6) lines in *Stipa* has probably given rise in part to the modified polyploid series reported for that genus.

In explaining the origin of the secondary basic number of x = 11 in the genus *Stipa*, AVDULOV (2) suggests that the number 44 may have occurred after the loss of chromosomes in tetraploid forms with a basic number of x = 12. In the present study the counts of 2n = 22 chromosomes for species of *Oryzopsis* which intergrade with *Stipa* indicate that the basic number of x = 11 might more probably have originated in a species with 2n = 24 chromosomes. However, a consideration of morphological characters together with chromosome numbers presents a more likely explanation for the origin of the basic number, x = 11.

As previously pointed out, in the section *Euoryzopsis* the four species *O. pungens*, *O. canadensis*, *O. exigua*, and *O. kingii* form a coherent group possessing stipoid characters, with *O. micrantha* somewhat detached from these owing to its possession of some *Piptatherum* characters. Counts of 2n = 22 chromosomes occur in the more stipoid group as well as in *O. micrantha*. A change from 2n = 24 to 2n = 22 in the x = 12 line leading to *Stipa* would not account for the *Piptatherum* characters of *O. micrantha*, nor would such a change in the *Piptatherum* line account for the *Stipa* characters of the other four species. It has been suggested, however, that the primary basic number in *Stipa* is x = 6 rather than x = 12, and this may apply as well to *Oryzopsis*. A single change from 2n = 12 to 2n = 10 in *Stipa*, with subsequent formation of allopolyploids, could account for both the origin of the basic number x = 11 and the combination of characters exhibited by *O. micrantha*.




FIGS. 81, 82.—Fig. 81, diagr m of natural groups in *Oryzopsis* showing probable interrelationships and general affinities to *Stipa*. Fig. 82, suggested pattern of differentiation of *Stipa* and *Piptatherum* genomes from common stock and their reduplication and recombination to give secondary basic and higher chromosome numbers associated with complexes of morphological characters in *Oryzopsis* and with generic characters of *Stipa*.

Assuming that the primary basic number for both *Stipa* and *Oryzopsis* is x = 6, the primitive genome may be designated as $6_x$ (fig. 82). By specialization in different directions, the *Stipa* genome $6_s$ and the *Piptatherum* genome $6_p$ may have been derived from the primitive one. Doubling of the chromosomes in each to give $6_s6_s$ and $6_p6_p$ would explain the counts of 2n = 24 cited for *Stipa sibirica* and for *O. miliacea*, *O. virescens*, and *O. holciformis* of the section *Piptatherum*. A single change in chromosome number in the $6_s$ genome could have produced a $5_s$ genome which may have had only a temporary existence or may have formed a third basic number in *Stipa*. The formation of one or more allopolyploids between the $6_s$ and $5_s$ genomes would account for the stipoid species *O. pungens* and *O. kingii* with 2n = 22 chromosomes now classed in *Oryzopsis*. Allopolyploidy between the $6_p$ and $5_s$ genomes would account for *O. micrantha* with 2n = 22 chromosomes and with a combination of *Stipa* and *Piptatherum* characters. Doubling of the chromosome number in $6_s5_s$ species of the x = 11 line could give rise to $6_s6_s5_s5_s$ polyploids, represented by 2n = 44 counts in *Stipa*. The higher allopolyploids, *O. racemosa* and *O. asperifolia*, may have been formed by combination of a $6_s5_s$ or $6_p5_s$ genome with a $6_p6_p$ genome to give $6_s5_s6_p6_p$ or $6_p5_s6_p6_p$. The origin of *O. hymenoides* may have been by the formation of a $6_s6_p$ combination, with subsequent doubling, or by direct formation of a $6_s6_s6_p6_p$ combination from the $6_s6_s$ and $6_p6_p$ lines. In the genus *Stipa* various combinations of $6_s$, $6_s6_s$, $6_s5_s$, and possibly $5_s$ genomes may account in part for its modified polyploid series. Certain counts in *Stipa* suggest that a third basic number of x = 5 may exist. For example, a simple backcross of a $6_s5_s$ genome to a $5_s$ genome and subsequent doubling would explain the count of 2n = 32 found in *S. webberi*.

It appears, therefore, that in the genus *Oryzopsis* the taxonomist has to contend not only with divergence of lines from a less specialized type but also with the convergence of these lines in allopolyploids which combine the characters of more highly specialized types. While all the hybrids between *O. hymenoides* and *Stipa* examined have been found to be sterile, the opportunity is nevertheless present from year to year for such perennial plants to form additional allopolyploids by doubling of their chromosome number and thus continue the process of reticulation between otherwise independent lines.

Such divergence and later convergence in the phylogenetic lines of a portion of the subtribe Stipeae have resulted in a large coherent group of species represented by the genus *Stipa* (fig. 81) and in a number of smaller peripheral groups which constitute the genus *Oryzopsis*. The groups in the latter genus are small aggregates of species together with the polyploids formed between these aggregates. In addition, hybrids are formed between one of these groups and the genus *Stipa*. Thus, an attempt to reduce the genera so as to include only species which are lineally related would still leave a few species which are intermediate between such genera and which do not among themselves constitute a homogeneous group.

The question of where to draw the boundary between the genus *Stipa* and the group of stipoid species of *Oryzopsis* is one which is frequently met among less specialized members of related genera. As judged from the chromosome counts which have been made, the line as drawn at present places the diploid

species with 2n = 22 chromosomes in *Oryzopsis* and the polyploids in *Stipa*. On the basis of morphological characters the separation is not clear, but to draw the line elsewhere would still not effect a distinct separation.

With the removal of *Stipa webberi* and the hybrids previously known as *Oryzopsis caduca* and *O. bloomeri* from the genus *Oryzopsis*, the latter is then left with a number of species whose interrelationships among themselves and whose affinities to *Stipa* can be interpreted.

## Summary

1. The Old World species *Oryzopsis miliacea*, *O. virescens*, *O. paradoxa*, *O. coerulescens*, and *O. holciformis*—which constitute most of the section *Piptatherum*—form a coherent group marked by specialization in characters which clearly distinguish them from the genus *Stipa*. On these characters *O. virescens* is most representative of the genus *Oryzopsis*, while *O. holciformis* shows the greatest specialization or departure from the generic characters of *Oryzopsis* and still greater divergence from the generic characters of *Stipa*. *O. miliacea* shows evidence of reduction from a specialized condition. *O. holciformis* and *O. miliacea* were found to have 2n = 24 chromosomes. Their idiograms are similar and both differ notably from the idiograms of other species of *Oryzopsis* studied. Previous counts of 2n = 24 have been reported for *O. miliacea* and *O. virescens*. It is concluded that the basic number for the section *Piptatherum* is x = 12 (or 6). This has been previously suggested as the basic number for the genus *Oryzopsis* and as the primary basic number for the genus *Stipa*.

2. The North American species *O. micrantha*, *O. pungeńs*, *O. exigua*, *O. canadensis*, and *O. kingii*—which constitute most of the section *Euoryzopsis*—form a group marked by specialization in characters which merge with the genus *Stipa*. *O. micrantha* is somewhat detached from the other species by its resemblance to the section *Piptatherum* on some characters, while the highly specialized *O. kingii* departs farthest from the generic characters of *Oryzopsis* and intergrades completely with *Stipa*. Chromosome counts of 2n = 22 were obtained for *O. micrantha*, *O. pungens*, and *O. kingii*. Their idiograms are similar, and all have longer chromosomes than in the Old World species. It is concluded that the basic chromosome number for the section *Euoryzopsis* is x = 11. A secondary basic number of x = 11 has been reported previously for *Stipa*. *O.*

3. The North American species *racemosa* and *O. asperifolia* combine morphological characters from the two lines in *Oryzopsis* with x = 12 (or 6) and x = 11 chromosomes. In size of plants and floral parts, they are larger than the species of either line. Both were found to have 2n = 46 chromosomes. The idiograms show that in range of chromosome lengths *O. racemosa* includes most of the range for the x = 12 (or 6) line plus most of the range for the x = 11 line. It is concluded that they are allopolyploids between those two lines. *O. racemosa* is referred to the section *Piptatherum*. *O. asperifolia* is the type of the section *Euoryzopsis*. Another species, *O. hendersoni*, may represent another such allopolyploid, as judged from morphological characters. It is referred to *Euoryzopsis*.

4. The North American species *O. hymenoides*, which is the only one in the section *Eriocoma*, resembles the genus *Stipa* in some features and *O. virescens* of the section *Piptatherum* in other features. A count of 2n = 48 chromosomes has been previously reported for *O.*

*hymenoides*. Its idiogram shows a range in chromosome lengths which greatly exceeds that for the x = 12 (or 6) line in *Oryzopsis*. It may be an allopolyploid between that line and the line in *Stipa* with a similar basic number or possibly a polyploid from the *Stipa* line alone. It hybridizes naturally with a number of species of that genus to produce sterile hybrids.

5. A variety of *O. hymenoides* with a contracted panicle is described.

6. *O. webberi* is transferred to *Stipa* on the basis of its close similarity to *S. pinetorum*. Both species have 2n = 32 chromosomes.

7. *O. bloomeri* and *O. caduca* are excluded from *Oryzopsis* on the basis of proof published elsewhere showing that they are sterile hybrids between *O. hymenoides* on the one hand and *S. occidentalis* and *S. viridula*, respectively, on the other hand.

8. It is concluded that the section *Piptatherum* and the genus *Stipa* in part represent divergent lines of specialization on a common primary basic number of x = 6 chromosomes and that these lines may have reticulated to form the polyploid section *Eriocoma*. The section *Euoryopsis* represents mainly the diploid species of a line that has tentatively become isolated from the x = 12 (or 6) lines by a change to a secondary basic number of x = 11. The latter line in turn has reticulated with the *Piptatherum* line to form allopolyploid species of *Oryzopsis* and with the *Stipa* line to contribute to the modified polyploid series reported for that genus.

9. It is suggested that the secondary basic number of x = 11 probably originated through a change from 2n = 12 to 2n = 10 within the *Stipa* line, with subsequent allopolyploidy between 2n = 10 individuals and the original 2n = 12 lines in both *Stipa* and *Piptatherum*.

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# INHERITANCE OF THE MAIN ANTHOCYANIN PIGMENTATION AND OF SOME OF ITS PATTERNS IN FLOWERS OF NEMESIA STRUMOSA[1]

HERBERT PARKES RILEY

The writer has shown that a number of genes are involved in the determination of flower color in *Nemesia strumosa* Benth., a South African member of the Scrophulariaceae (**23, 24, 25**). The white-flowered type can be regarded as the standard. It has a two-lipped corolla whose limb is white (or perhaps ivory, comparable with LAWRENCE'S [**14**] ivory-colored dahlia). The lower part of the short tube is orange and frequently contains deep purple spots which vary greatly in number and size and which frequently are so dark-hued as to appear black. These purple spots may not be present, but, if they are, they are accompanied by a deep purple spot on the upper lip just above the stamens.

One of the main variants is the orange type, which differs from the white-flowered form by the presence of a deep orange-yellow sap pigment in the epidermal cells of the inner part of the corolla limb. Orange is a simple dominant to white and is determined by gene *O*. The orange type can be modified by other genes, of which two have been analyzed. The recessive gene for pale-upper, *p*, causes the upper lip of orange-colored flowers to be considerably lighter in hue, while the lower lip is unaffected. The recessive gene for buff, *bu*, modifies the lower lip to Buff Yellow[2] and the upper lip to Sulphur Yellow with areas of Buff Yellow. Gene *bu* is linked with the self-sterility alleles, with about 5% crossing over, and is epistatic to gene *p*.

Three recessive genes are responsible for the presence of yellow pigmentation in *oo* plants. Gene *gl* (glass-green) produces a color in the limb which is usually close to Sea-foam Yellow or Pale Glass-Green, but *glgl* are sometimes so pale as to be difficult to distinguish from white. A deeper yellow color is produced by the recessive gene, *y* (yellow). Yellow plants are not so deep as orange and may vary from slightly darker than glass-green at the one extreme to almost buff at the

[1] This paper is one of a series on the genetics of the genus *Nemesia*. Some of the data were collected at Princeton University and as a National Research Council fellow in the Biological Sciences at the Bussey Institution of Harvard University. Later families were grown at the University of Washington (Seattle) and at the University of Kentucky.

[2] Throughout this paper, the names of colors which are capitalized are those listed as such in RIDGWAY (**21**).

other. Because of this variation, and because of the resemblance to buff, it has presented some difficulty. The yellow-spot gene (*ys*) produces a number of dots or streaks radiating from a small yellow area on the lower lip, near the throat.

As in the case of many other plants, one of the common flower-color types in *N. strumosa* is the "colored" type that contains anthocyanin pigment in the cells of the corolla and which appears as some shade of red or purple. That this type is due to a single dominant gene, *C*, has been previously mentioned (**23**).

Anthocyanin pigmentation was one of the first characters studied in genetics, being one of the characters in MENDEL'S (**18**) original experiments. Experiment 3 of his paper deals with the color of the seed coat, flowers, and stems of the pea. In plants with the dominant gene, the seed coats are gray, gray-brown, or leather-brown, and opaque; the standards of the flowers are violet and the wings are purple; while the stems in the axils of the leaves are of a reddish tint. In the homozygous recessive, the seed coats are transparent, the flowers are white, and there is no red color in the stems. In some experiments with plants other than the pea, MENDEL crossed the white-flowered *Phaseolus nanus* with the purple-red-flowered *Ph. multiflorus*. The first species also had white seeds, while the latter had red seeds with black flecks and splashes. This color difference was not the result of a single gene, and MENDEL considered that the result "might probably be explained by the law governing *Pisum* if we might assume that the colour of the flowers and seeds of *Ph. multiflorus* is a combination of two or more entirely independent colours, which individually act like any other constant character in the plant." Anthocyanin pigmentation was also reported in a number of other plants in 1900 and 1901. For example, the dominance of colored to noncolored was demonstrated in *Agrostemma*, *Atropa*, *Belladonna*, *Clarkia pulchella*, *Hyoscyamus* hybrids, *Lychnis*, *Papaver somniferum*, *Polemonium coeruleum*, *Trifolium pratense*, and *Veronica longifolia* by DE VRIES (**36**); in *Anemone* hybrids by HILDEBRAND (**9**); in *Epilobium angustifolium* and in *Matthiola* hybrids by CORRENS (**5, 6**); and in beans by VON TSCHERMAK (**34**). HILDEBRAND (**9**), however, found that a cross between a light blue *Anemone angulosa* and a dark blue *A. hepatica* produced an intermediate color in the $F_1$, while DE VRIES (**36**) found that in *Coreopsis tinctoria* yellow was dominant to brown and postulated that the yellow-flowered type possessed a gene that inhibits anthocyanin production.

It has been found frequently that the formation of anthocyanin pigment depends upon the simultaneous presence of more than one gene, and that these act as complementary genes. Such a situation has been demonstrated in a number of plants, including *Antirrhinum majus* (**3, 4**), *Campanula medium* (**13**), *Cattleya*, *Cypripedium*, and *Dendrobium* (**10**), *Dolichos lablab* (**8**), *Ipomoea purpurea* (**1**), *Lathyrus odoratus* (**2**), *Linum usitatissimum* (**33**), *Lychnis* (**32**), *Matthiola incana* (**35, 28**), *Mirabilis jalapa* (**17**), *Pharbitis nil* (**11**), *Phaseolus vulgaris* (**31**), *Phlox drummondii* (**12**), and *Solanum tuberosum* (**26**). In a number of instances, the two complementary genes have been designated *C* and *R*. Complementary genes for anthocyanin pigment have been found in so many different plants that SANSOME and PHILP (**27**) conclude, "It is, however, significant that the *CR* type of inheritance of colour is widespread among plants and is prob-

ably connected with anthocyanin pigments in almost every case."

In some plants the situations may be somewhat different, as in LAWRENCE'S (14) study of *Dahlia variabilis*, where gene *A* produces a relatively pale color while *B* produces a deeper color; both genes appear to be cumulative in their action.

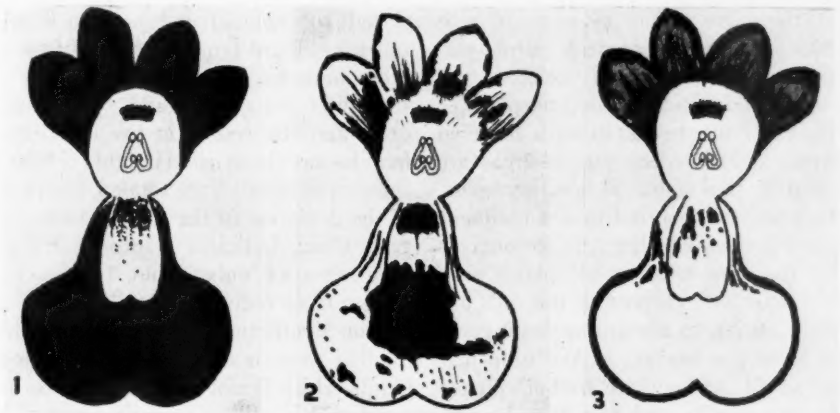
In many plants both anthocyanin and yellow sap pigments may be present. In some of these cases it has been demonstrated that the anthocyanin color is red if a yellow pigment is present but magenta if the background is ivory rather than yellow. In the snapdragon, for example, gene *Y* produces yellow color owing to the presence of a glucoside of luteolin in the corolla lips, while gene *I* produces an ivory color owing to a glucoside of apigenin. In yellow-flowered plants, both glucosides are present. Apparently gene *I* acts only in the presence of *Y*, for *yyI-* and *yyii* are white and have no flavone pigment (**37, 38, 39, 19, 20, 29**). When anthocyanin pigment is present in *Y-ii* (yellow) plants, its color is crimson, but if it is present in *Y-I-* (ivory) plants, it is magenta. On the other hand, in Indian cotton, LEAKE (15) failed to distinguish between anthocyanin plus yellow and anthocyanin plus white. He found that the flowers of *Y-RR* plants were red, those of *Y-Rr* were red on yellow, while those of *yyRR* and *yyRr* were red on white.

LAWRENCE'S (14) observations on *Dahlia variabilis* are interesting, as the background may be yellow, cream, ivory, or white, because two genes for anthocyanin are present, because each appears to act cumulatively, and because each is tetrasomic since the species is an octoploid. The background is determined principally by genes *Y* and *I*. Gene *Y* produces yellow pigment and is completely epistatic to *I*, so that all *YI* and *Yi* combinations are yellow; gene *Y* is tetrasomic. Gene *I*, which is disomic, produces an ivory flower in the absence of *Y*. All *yi* plants are white. In addition, genes which inhibit the *Y* gene may be present and produce a cream type. The *B* gene produces a deeper anthocyanin pigment than does *A*, but both genes tend to be on the magenta side with ivory and on the orange-scarlet side with yellow. Thus, *A* in the simplex condition is pale magenta with ivory and apricot with yellow, while *B* simplex is, respectively, purple and scarlet. Additional *A* and *B* genes (or both) deepen the color, but in all cases the colors remain within the ranges. Unanalyzed, modifying genes also vary the shade and may also affect the intensity of the color. For example, in some families the magenta class (*Aby*) is "easy to score and very distinct"; but when the Union Jack variety is one of the parents, there is a considerable deficiency of magentas but an excess of the deeper rosy-purple. Similarly, in apricot flowers, if there is a mutation of the *Y* gene to *y* so as to produce ivory sectorial chimaeras, the magenta color of the sector is considerably deeper than the apricot regions next to it, suggesting that modifying genes have a different effect on *AI* from that produced on *AY* regions. Another factor that influences the color of anthocyanin types is the intensity of the yellow background color. If it is a good yellow, certain genotypes may appear scarlet as expected; if, however, yellow-inhibiting genes are present and the background is cream, the same genotypes (exclusive of the inhibitor) may appear purple and resemble a plant with the same *A* and *B* gene combination but with an ivory background. Since certain anthocyanin-gene combinations may give the same result with *YI* and with

*Yi* plus a *Y* inhibitor, the scoring of the background in "colored" plants is often subject to considerable error.

### Observations

The materials used for this study were the same as those described previously (25). In fact, many of the families reported in that paper were the same as those involved in this study.




FIGS. 1–3.—Diagrams of flowers of *Nemesia strumosa*. Each flower is cut along both sides of tube and then flattened out (causing some distortion of the tube but little of the lips). Fig. 1, self-colored purple or red type; *C Sp Gr Ro*. Fig. 2, splotched; *C sp Gr Ro*. Fig. 3, red-outline; *C Sp Gr ro*. In each flower, blackened areas show distribution of anthocyanin (red or purple) pigmentation, except that black areas and dots in rear of lower part of tube and black "eyebrow" over stamens represent very deep purple pigmentation caused by another gene. For comparison with standard type, see 25.

### COLORED (C)

The white-flowered type which has been taken as the standard in this work has been described previously. In the colored type the inner part of the corolla limb is a shade of purple or red instead of white, but otherwise the white and colored types are similar with respect to the inner part of the corolla (fig. 1). As in some other plants, the tendency is toward the red shades if the background is orange owing to the dominant gene *O*, but magenta or purplish if the background is white (*oo*). Owing probably to the presence of numerous modifying genes, there is some overlapping of phenotypes, and an attempt to determine the background by examining the color of flowers with anthocyanin pigmentation is subject to considerable error. Probably the most frequent colors of the anthocyanin in combination with white are Pomegranate Purple or Amaranth Purple, while the most frequent anthocyanin-orange types are Carmine or Oxblood Red. However, other colors frequently found on a white background are Tyrian Rose and Rose Color and on an orange background are Bordeaux and Garnet Brown.

In Pomegranate Purple plants, the cells of the inner epidermis of the limb of the corolla contain a purplish red pigment in solution in the cell sap. When ammonia is added, most of the cells turn olive brown, and many turn olive green, while a few become bright green. When

hydrochloric acid is added, the colored cells turn bright red. Further chemical tests have not been made, but it is probable that this pigment is the same as or is closely related to the cyanidin 3-bioside reported for pink and crimson *Nemesia* hybrids by SCOTT-MONCRIEFF (**30**). In the throat most of the cells are orange, but there are numerous areas of cells which contain a very deep purple pigment, and an occasional "colored" cell is bright red instead of deep purple. The throat is similar to that in white-flowered plants, and the deep purple areas are probably the result of another gene, which will be reported in a subsequent paper. In the area where the lip connects with the tube, in "colored" plants on a white background most of the cells are purple, owing to the anthocyanin gene, but some are orange; in the tube just back of this region there are both purple and orange cells, with the latter becoming more frequent the farther they are from the lip. These purple cells are not to be confused with the deep purple, almost black, cells of the "black" spots of the tube. In the throat region all the long hairs are yellow, even in purple ("colored" as distinguished from "black") areas.

When a purple-flowered plant is placed in ammonia, dark green spots soon appear in the purple regions, and the white (or ivory) regions at the sides of the throat turn yellow. When the epidermis is stripped off, the white regions beneath turn yellow. The lips continue to turn green and finally become very dark. In sulphur dioxide the lips fade to Rose Color but do not seem to fade out entirely. In hydrochloric-acid vapor, the lips turn close to Nopal Red.

That colored is dominant to noncolored is observed from a number of crosses in both the old and new material. In two families, crosses between two colored plants yielded 140 colored and no noncolored. In twenty-two families from crosses between two noncolored, there were no colored plants and 1146 noncolored. Crosses between a noncolored and a colored that must have been homozygous were made in three cases and produced 190 colored and no noncolored offspring. Eight families resulted from a cross between two heterozygotes. The total was 403 colored and 172 noncolored, and the results in the individual families have been listed in table 1. When these eight families are totaled, the ratio of the deviation to the standard error is 2.72, which indicates a probability of occurrence of only about 0.69%, although in no individual family is the deviation significant. In each of the eight families there is a deficiency of colored plants which is not large enough to be significant in any one family but which is significant when the eight families are combined. The reason for this slight deficiency of colored plants cannot readily be explained. Whenever disturbed ratios occur in plants which are self-sterile, linkage with the self-sterility alleles must be considered as a possible explanation. While such an explanation will fit certain of the families in table 1 it will not fit others, and if all the families in tables 1 and 2 are diagrammed, it becomes apparent that linkage with the *s* genes is not the explanation. For example, family 3209 arose from the cross 3112(11) × 3211(4); it has been shown (**22**) that the self-sterility alleles of the parents are $s^2s^4$ and $s^1s^2$. The female parent was white, so that, if there were linkage, its genetic constitution would be $s^2c/s^4c$. Plant 3209(3) was purple and $s^1s^2$, so it could only be $s^1C/s^2c$, since its $s^2$ gamete must have come from the noncolored female parent. When this is crossed as a

male on to 3211(4), a *Cc* plant with the constitution $s^2s^3$, there is a decided deficiency of colored plants in the offspring (family 3374). On the basis of linkage between *c* and *s*, however, an excess of coloreds would be expected, because the $s^2$ served ratio of 26:29 shows no significant deficiency. Other similar examples might be cited to show that the consistent slight deficiency of *C* plants in the eight $F_2$ families is not due to linkage of *C* with the self-sterility alleles.

TABLE 1

RATIO OF COLORED TO NONCOLORED IN EIGHT FAMILIES, OF WHICH BOTH PARENTS WERE HETEROZYGOUS FOR *C*

| FAMILY | PARENTS | OBSERVED | | EXPECTED | | Dev./S.E. |
|---|---|---|---|---|---|---|
| | | *C* | *c* | *C* | *c* | |
| 3004 | 2901(7) × (13) | 70 | 25 | 71.25 | 23.75 | 0.30 |
| 3012 | 2901(13) × (10) | 61 | 31 | 69.0 | 23.0 | 1.93 |
| 3211 | 3111(4) × (5) | 31 | 16 | 35.25 | 11.75 | 1.43 |
| 3214 | 3111(5) × (4) | 17 | 7 | 18.0 | 6.0 | 0.47 |
| 3226 | 3103(5) × (3) | 44 | 22 | 49.5 | 16.5 | 1.56 |
| 3366 | 3206(2) × (1) | 52 | 20 | 54.0 | 18.0 | 0.56 |
| 3372 | 3209(3) × 3226(7) | 60 | 21 | 60.75 | 20.25 | 0.19 |
| 3374 | 3111(4) × 3209(3) | 71 | 30 | 75.75 | 25.25 | 1.09 |
| Total | | 406 | 172 | 433.50 | 144.50 | 2.64 |

TABLE 2

RATIO OF COLORED TO NONCOLORED IN EIGHT TESTCROSS FAMILIES IN WHICH FEMALE IS RECESSIVE AND MALE HETEROZYGOUS. IN THE FIRST THREE FAMILIES, BACKGROUND IS ORANGE; IN THE OTHER FIVE IT IS WHITE

| FAMILY | PARENTS | SELF-STERILITY ALLELES | OBSERVED | | EXPECTED | | Dev./S.E. |
|---|---|---|---|---|---|---|---|
| | | | *C* | *c* | *C* | *c* | |
| 3103 | 3001(6) × (22) | $s^xs^y \times s^ys^z$ | 35 | 44 | 39.5 | 39.5 | 1.01 |
| 3225 | 3103(1) × (5) | $s^ys^z \times s^xs^z$ | 14 | 16 | 15.0 | 15.0 | 0.36 |
| 3361 | 3103(52) × (4) | $s^xs^z \times s^ys^z$ | 15 | 7 | 11.0 | 11.0 | 1.70 |
| 3206 | 3112(10) × 3111(4) | $s^2s^3 \times s^1s^2$ | 6 | 3 | 4.5 | 4.5 | 1.00 |
| 3209 | 3112(11) × 3111(4) | $s^3s^4 \times s^1s^2$ | 5 | 7 | 6.0 | 6.0 | 0.58 |
| 3217 | 3111(3) × (4) | $s^1s^3 \times s^1s^2$ | 28 | 12 | 20.0 | 20.0 | 2.50 |
| 3367 | 3209(1) × (3) | $s^1s^4 \times s^1s^2$ | 26 | 29 | 27.5 | 27.5 | 0.40 |
| 3406 | 3206(6) × (1) | $s^1s^3 \times s^1s^2$ | 16 | 6 | 11.0 | 11.0 | 2.13 |
| Total | | | 145 | 124 | 134.5 | 134.5 | 1.28 |

gamete would be eliminated from the male parent, and this is coupled with the *c* gene. Likewise, family 3367 arose from the cross 3209(1) × 3209(3) and should show a significant deficiency of *C* plants when compared with a 1:1 ratio. The observed ratio of 26:29 shows no significant deficiency. Other similar examples might be cited to show that the consistent slight deficiency of *C* plants in the eight $F_2$ families is not due to linkage of *C* with the self-sterility alleles.

In thirty-two testcross families the combined ratio was 687 colored: 648 noncolored, and only two families deviated significantly from expectation. In eight of the families the male was heterozygous and there was a common self-sterili-

ty allele, and six agreed well (table 2) with expectation on the basis of no linkage. Four showed a deficiency of *C* and four of *c* plants.

The dominant gene for the main anthocyanin pigmentation will be designated *C*. This pigmentation has been referred to as the *main* anthocyanin pigmentation, because several other cases of anthocyanin pigmentation due apparently to entirely different genes have been found and will be reported in a subsequent paper.

It has been shown that, in a number of plants, anthocyanin flower color is the result of two complementary genes, usually designated *C* and *R*. Either this is not the case in *N. strumosa* or the original plants were all homozygous for one of the dominant color genes. At any rate, there is no evidence for complementary genes, for no families from two colored plants segregated into ratios that approached 9:7, and there were no cases where two noncolored plants produced colored when crossed.

It has been stated that it is difficult in some families to determine from the color of the anthocyanin pigment whether the background is orange or white. In a few families which perhaps lack certain modifying genes this distinction can be made easily. For example, in family 3010, from the cross *CcOo* × *CCOo*, there are 73 red :28 purple. The red plants are Ox-blood Red, Burnt Lake, Bordeaux, Scarlet Red, and Coral Pink, and the purple plants are Rose Color. This family is one of the few easy ones to score. The interaction of the anthocyanin pigmentation with such types as buff, pale-upper, yellow, glass-green, or yellow-spot has not been tested.

There is no evidence for linkage between the *C* and *O* genes and two families appear to indicate that these genes are not linked. Family 3004 came from the cross *CcOo* × *Ccoo* and would be expected to segregate into a ratio of 6 colored : 1 orange : 1 white if there is no linkage. The observed ratio is 70 : 10 : 15, which does not deviate significantly from the expected. The parents of family 3006 were *ccoo* and *CcOo*. This should segregate into 2 colored : 1 orange : 1 white, and the observed ratio of 54 : 22 : 19 agrees with the assumption of no linkage.

It has not been possible in *C* plants with an orange background to determine whether they are also *Bu* or *bu*. Therefore, to determine whether there is any linkage between *C* and *bu*, the ratio of *Bu* to *bu* in noncolored plants must be examined; it must not be 3 : 1 or 1 : 1. For example, in the testcross *CcBubu* × *ccbubu*, a ratio of 2 colored : 1 orange : 1 buff is to be expected if there is no linkage, while the ratio will be 1 colored : 1 buff if linkage is complete and is in the coupling phase. If there is some crossing over, half the offspring will be colored and of the remainder there will be some orange (nonbuff), but the percentage of orange among the noncolored will not equal 50%. Similarly, if the original cross is in the repulsion phase, the ratio will be 1 colored : 1 orange (nonbuff) if linkage is complete; if there is some crossing over, some buff plants will be present, but they will not equal 50% of the noncolored. If *bu* and *C* are completely linked and if one parent is homozygous for both *bu* and *c* while the other is heterozygous for both and has the two dominant genes on one chromosome and the two recessives on the other, the noncolored offspring will all be buff. The same result will be obtained if *bu* is not linked with *C*, but if it is completely linked with the self-sterility alleles and if the female parent is homozygous for both *bu* and *c*

while the male is heterozygous for both and the *Bu* gene in the male is on the same chromosome as the common self-sterility allele. Thus the cross *cbu/cbu* × *CBu/cbu* will give the same ratio of *Bu* to *bu* in *cc* plants as will the cross *cc* $s^1bu/s^2bu \times Cc\ s^1Bu/s^3bu$. Similarly, the cross *cbu/cbu* × *Cbu/cBu* will yield the same results as the cross *cc* $s^1bu/s^2bu \times Cc\ s^1bu/s^3Bu$. In such cases it is impossible to determine from the *Bu*:*bu* ratio alone whether *bu* is linked with *C* or with *s*. On the other hand, in the reciprocal crosses there is no evidence of linkage if *bu* is linked with *s*, but the ratios will be the same if *bu* and *C* are linked no matter in which direction the cross is made. Although several families indicate nothing more than that *bu* is linked with either *C* or *s*, several others indicate that the linkage is with the self-sterility alleles—as has been suggested previously (**24, 25**). In family 3362 the ratio is disturbed, although there is no *C* gene in either parent, so that it could not be due to linkage with *C*. Family 3004 came from the cross *CcBubuOo* × *Ccbubuoo* and segregated into 70 colored : 6 orange (nonbuff) : 4 (orange) buff : 15 white. The ratio of 1 nonbuff : 1 buff is expected if *bu* is linked with *s*, since the male is recessive, but it would not be expected if *bu* were linked with *C*. There is, therefore, no evidence that *C* is linked with *bu*.

Since genes *P* and *p* cannot be differentiated in *C* plants, any linkage between *p* and *C* must be determined from the *P*:*p* ratio in the *cO* plants of families which are segregating for *C*. If *p* and *C* are linked, the ratio of *P* to *p* should not be 1:1 or 3:1 in backcross or $F_2$ families in which some of the plants are *C*. In table 1 of a previous paper (**25**), eight families segregating for *p* are listed. All but family 3362 are also segregating for *C*. Of the seven families which segregate for *C*, only one deviates significantly from a 1:1 or 3:1 ratio, and this is a family with only ten *cc* plants. The evidence from these families seems to indicate clearly that *p* is not linked with *C*.

Just as possible linkage between *C* and *bu* or *p* must be tested from the *Bu* : *bu* and *P* : *p* ratios in *c* plants, any linkage between *C* and genes *y*, *gl*, or *ys* must be determined solely from the ratios of these yellow genes and their dominant alleles in the *c* plants of families segregating for *C*. There is only one family that offers any evidence in regard to possible linkage between *C* and *y*. Family 3012 arose from a cross between two *CcYy* plants. As the ratio of white : yellow does not deviate significantly ($\chi^2 = 0.52$; df = 1; P = 30–50%), there is no reason to assume that these genes are linked. Six families have at least one parent heterozygous for both *C* and *gl* and might therefore be used to test possible linkage between these genes. Five families (table 3) agree with the assumption of no linkage, and the sixth does not deviate too badly, although significantly. Three families suggest no linkage between *C* and *ys*, while the fourth (table 3) deviates significantly. In view of the difficulty of scoring *gl* and *ys*, however, it cannot be said that there is any evidence for linkage between *C* and either of these genes.

### Splotched (sp) and granular (gr)

Splotched and granular are both modifications of colored and act only on *C* plants. In the earlier material they were found only in the line of plants which had an orange background, except that they were possibly present in families 3005 and 3013, two of the $F_1$ families that were not used to produce further generations. They have not been tested in the new material. Families 3005 and 3013 were very complicated, segregating for a

large number of genes, and it is possible that some phenotypes that resembled those produced by the splotched and granular genes were actually the result of other genes. The patterns in these families were very difficult to score.

In general, it is not an easy matter to score and to analyze the patterns which appear on *C* plants. The splotched and granular types are fairly clear, but some of the others have been impossible to study. This is especially true when sev-

TABLE 3

RATIOS OF NONGLASS-GREEN: GLASS-GREEN AND NONYELLOW-SPOT: YELLOW-SPOT IN FAMILIES ALSO SEGREGATING FOR *C*. IN FIRST SIX FAMILIES, RATIO OF DEVIATION TO STANDARD ERROR REPRESENTS *Gl*: *gl*; IN THE LAST FOUR IT STANDS FOR *Ys*: *ys*

| Family | Genotypes of parents | Colored | Orange | White | Glass-green | Yellow-spot | Dev. / S.E. (if no linkage) |
|---|---|---|---|---|---|---|---|
| 3004..... | *Cc Glgl* × *Cc Glgl* | 70 | 10 | 13 | 2 | ..... | 1.04 |
| 3006..... | *cc glgl* × *Cc Glgl* | 53 | 22 | 10 | 9 | ..... | 0.23 |
| 3111..... | *Cc Glgl* × *cc Glgl* | 16 | ..... | 10 | 4 | ..... | 0.31 |
| 3215..... | *Cc Glgl* × *cc Glgl* | 18 | ..... | 17 | 5 | ..... | 0.25 |
| 3374..... | *Cc Glgl* × *Cc Glgl* | 71 | ..... | 26 | 4 | ..... | 1.48 |
| 3375..... | *Cc Glgl* × *cc Glgl* | 29 | ..... | 26 | 2 | ..... | 2.18 |
| 3209..... | *cc Ysys* × *Cc Ysys* | 5 | ..... | 6 | ..... | 1 | 0.66 |
| 3211..... | *Cc Ysys* × *Cc Ysys* | 31 | ..... | 13 | ..... | 3 | 0.58 |
| 3213..... | *Cc Ysys* × *cc Ysys* | 12 | ..... | 14 | ..... | 2 | 1.16 |
| 3216..... | *Cc Ysys* × *cc Ysys* | 9 | ..... | 2 | ..... | 7 | 3.65 |

eral pattern genes are present in the same plant. In the splotched type, the anthocyanin pigmentation is not spread evenly over the entire limb of the corolla but is dispersed in a number of lines and splotches, much as in figure 2. As might be expected with a pattern of this sort, there is considerable variation in its details in different plants, and even in different flowers on the same plant. While this is true, the splotched type can usually be recognized rather readily, provided that too many modifying genes are not present. The granular type is more like the normal colored type, in that the color is much more evenly and regularly distributed than it is in splotched plants. There are, however, numerous small white dots that appear over the entire colored area.

Splotched is apparently recessive to self color, but the data in support of this are not numerous. Family 3009 arose from a nonsplotched colored and a noncolored which presumably was heterozygous for splotched. It segregated into 8 colored nonsplotched : 2 colored splotched : 9 noncolored. Family 3004 arose from a cross between a colored nonsplotched plant which had a red outline and a colored nonsplotched. It segregated into 20 colored orange nonsplotched : 2 colored orange splotched : 48 colored nonorange : 10 orange : 15 white. The smaller number of splotched in both families is a good indication of the recessive nature of the gene, a supposition which is corroborated by families 3001 and 3103 (table 4). The symbol for the splotched gene shall be *sp*. Since *Sp* and *sp* cannot be distinguished in *c* plants, the *c* gene is epistatic to the splotched gene. Family 3004 indicates that in *C* plants *o* is also epistatic to *Sp* and *sp*.

This, however, needs further corroboration.

Granular also appears to be recessive to self color. Family 3226 arose from two colored, nongranular plants which were apparently heterozygous for both *C* and the granular gene. This family segregated into 31 colored nongranular : 13 colored granular : 22 noncolored. In family 3001, of which the parents were a noncolored female apparently heterozygous for granular and splotched and a male heterozygous for colored, granular, and splotched, there were 8 self-colored, 1 splotched, and 2 granular. Family 3013 segregated into 6 colored orange : 8 splotched : 3 granular : 23 colored on a white background. The fact that granular plants form such a small percentage of the individuals in these three families suggests that the granular type is the result of a recessive gene. One family (3223) gives a peculiar ratio that appears to contradict this (table 4) and must remain unexplained. Six testcross families totaled 49 colored nongranular and 52 colored granular (table 4). Granular appears to be a simple recessive to nongranular and will be designated by the symbol *gr*.

When two recessives such as *sp* and *gr* both affect the same part of the plant, the question should be raised whether *sp gr* plants constitute a different phenotype from *Sp gr* or *sp Gr* or whether they are indistinguishable from one of these types. Four families throw light on this question, but a discussion of two of them will be postponed until the question of linkage of these genes with *C* is discussed. Famliy 3013 arose from the cross *Cc oo Spsp Grgr* × *CC OO spsp Grgr* and segregated into 6 *COSpGr* : 8 *COspGr* : 3 *COSpgr* : 23 *Co*. If *sp* is epistatic, the expected frequencies of the first three terms of the ratio (excluding the *Co*

TABLE 4

FREQUENCIES OF COLORED (NONSPLOTCHED, NONGRANULAR), SPLOTCHED, GRANULAR, ORANGE, PALE-UPPER, AND BUFF TYPES IN ELEVEN FAMILIES SEGREGATING FOR SPLOTCHED, GRANULAR, OR BOTH

| Family | Parents | Genotypes of parents | Colored | *sp* | *gr* | Orange | *p* | *bu* |
|---|---|---|---|---|---|---|---|---|
| 3001 | 2901(1) × (7) | *cc Spsp Grgr Bu/* × *Cc Spsp Grgr Bu/* | 8 | 1 | 2 | 4 | 7 | 6 |
| 3101 | 3001(6) × (12) | *cc Spsp grgr* × *Cc spsp Grgr* | 6 | 14 | 10 | 14 | 8 | 0 |
| 3103 | 3001(6) × (22) | *cc Spsp grgr s*$^{s}$*bu/s*$^{p}$*Bu pp* × *Cc Spsp Grgr s*$^{p}$*Bu/s*$^{s}$*bu P*$^{p}$ | 18 | 4 | 13 | 13 | 8 | 23 |
| 3105 | 3001(8) × × (22) | *cc SpSp grgr s*$^{s}$*bu/s*$^{p}$*Bu* × *Cc Spsp Grgr s*$^{p}$*Bu/s*$^{s}$*bu* | 11 | 0 | 13 | 10 | 0 | 10 |
| 3106 | 3001(22) × (8) | *Cc Spsp Grgr s*$^{p}$*Bu/s*$^{s}$*bu* × *cc SpSp grgr s*$^{s}$*bu/s*$^{p}$*Bu* | 9 | 0 | 12 | 3 | 0 | 6 |
| 3223 | 3103(3) × (52) | *Cc SpSp Grgr s*$^{p}$*Bu/s*$^{s}$*Bu P*$^{p}$ × *cc Grgr s*$^{s}$*bu/s*$^{s}$*bu P*$^{p}$ | 5 | 0 | 10 | 16 | 9 | 0 |
| 3225 | 3103(1) × (5) | *cc SpSp s*$^{p}$*bu/s*$^{s}$*bu P*$^{p}$ × *Cc spsp Grgr s*$^{p}$*Bu/s*$^{s}$*bu P*$^{p}$ | 8 | 0 | 6 | 7 | 9 | 0 |
| 3226 | 3103(5) × (3) | *Cc spsp Grgr s*$^{s}$*Bu/s*$^{s}$*bu P*$^{p}$ × *Cc SpSp Grgr s*$^{p}$*Bu/s*$^{s}$*Bu P*$^{p}$ | 31 | 0 | 13 | 20 | 2 | 0 |
| 3301 | 3103(52) × (4) | *cc Grgr s*$^{s}$*bu/s*$^{s}$*bu P*$^{p}$ × *Cc SpSp grgr s*$^{p}$*Bu/s*$^{s}$*bu P*$^{p}$ | 7 | 0 | 8 | 7 | 0 | 0 |
| 3411 | 3103(4) × (52) | *Cc SpSp grgr s*$^{p}$*Bu/s*$^{s}$*bu P*$^{p}$ × *cc Grgr s*$^{s}$*bu/s*$^{s}$*bu P*$^{p}$ | 5 | 0 | 0 | 5 | 3 | 1 |
| 3373 | 3220(7) × (1) | *Cc Spsp Grgr s*$^{p}$*Bu/s*$^{s}$*bu* × *cc Spsp grgr s*$^{s}$*Bu/s*$^{p}$*Bu* | 7 | 0 | 13 | 13 | 0 | 0 |

types) are 6.4, 8.5, and 2.1, while the expected frequencies if *gr* is epistatic are 6.4, 6.4, and 4.2. In the first case, chi-square is 0.45, which—with three degrees of freedom—indicates a probability of 70–80%, while in the latter, chi-square is 0.77 and P is 50–70% (table 5). In family 3103, the cross is *cc Spsp grgr* × *Cc spsp Grgr* and the observed ratio was 6 colored : 14 splotched : 10 granular : 22 noncolored. On the basis of the epistasis of *sp*, the expected ratio (excluding noncolored) is 7.5 : 15 : 7.5, but if *gr* is epistatic, the ratio becomes 7.5 : 7.5 : 15. As shown from table 5, there is a much better agreement if it is assumed that *sp* is epistatic to *gr*.

The possible connection between the *sp* and *gr* genes and *bu* and *p* must be considered. Are either *sp* or *gr* merely the expressions of *bu* and *p* on a *C* rather than on a *c* background? Families 3225 and 3226 might indicate that granular was merely the expression of the pale-upper gene on a *C* background, since the first family is segregating into 1 *Gr* : 1 *gr* and 1 *P* : 1 *p*, while the second is segregating into a 3 : 1 ratio in each case. However, a study of 3105 and 3106 (table 4) shows a segregation into 1 *Gr* : 1 *gr*, but no *p* plants. This would tend to show that *gr* and *p* are not the same genes. Similarly, in these two families there is a segregation into 1 *Bu* : 1 *bu* (because of the elimination of $s^y$ male gametes), which might indicate that *gr* and *bu* were identical; but the first two families disprove that, since they are segregating for *gr* but not for *bu*. Families 3361 and 3373 support these suggestions, since they are both segregating for *gr* but for neither *p* nor *bu*. There is some evidence also to indicate that *sp* is not identical with *p* or with *bu*. Family 3101 is segregating into 1 *Sp* : 1 *sp* but has no buff plant, while in 3411 there are *bu* but no

TABLE 5

SEGREGATION INTO COLORED (*C*), SPLOTCHED (*sp*), AND GRANULAR (*gr*) ON ORANGE BACKGROUND, AND PROBABILITY ACCORDING TO EPISTASIS OF *sp* AND OF *gr*, AND ALSO (IN TWO FAMILIES) ACCORDING TO NO LINKAGE BETWEEN *C* AND *sp* AND ACCORDING TO LINKAGE WITH 20% CROSSING OVER. COLORED NONORANGE AND NONCOLORED TYPES NOT INCLUDED IN CALCULATION OF CHI-SQUARE VALUES

| FAMILY* | OBSERVED | | | | | CHI-SQUARE VALUES | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Linkage | | No linkage | |
| | *CO* | *sp* | *gr* | *Co* | *c* | *sp* epistatic | *gr* epistatic | *sp* epistatic | *gr* epistatic |
| 3001.... | 8 | 1 | 2 | 0 | 17 | 0.16 (90–95%)† | 0.32 (80–90%) | 1.68 (30–50%) | 1.25 (50–70%) |
| 3103.... | 18 | 4 | 13 | 0 | 44 | 0.87 (50–70%) | 4.37 (10–20%) | 29.05 (less than 1%) | 4.45 (10–20%) |
| 3013.... | 6 | 8 | 3 | 23 | 0 | Cannot test linkage | | 0.45 (70–80%) | 0.77 (50–70%) |
| 3101.... | 6 | 14 | 10 | 0 | 22 | Cannot test linkage | | 0.93 (50–70%) | 7.60 (2–5%) |

* Genotypes of parents.—
3001: *cSp/csp Grgr* × *CSp/csp Grgr*
3103: *cSp/csp grgr* × *CSp/csp Grgr*
3013: *CSp/cSp Grgr* × *Csp/Csp Grgr*
3101: *cSp/csp grgr* × *Csp/csp Grgr*

† Figures in parentheses indicate probabilities for two degrees of freedom.

The possible connection between the *sp* and *gr* genes and *bu* and *p* must be considered. Are either *sp* or *gr* merely the expressions of *bu* and *p* on a *C* rather than on a *c* background? Families 3225 and 3226 might indicate that granular was merely the expression of the pale-upper gene on a *C* background, since the first family is segregating into 1 *Gr* : 1 *gr* and 1 *P* : 1 *p*, while the second is segregating into a 3 : 1 ratio in each case. However, a study of 3105 and 3106 (table 4) shows a segregation into 1 *Gr* : 1 *gr*, but no *p* plants. This would tend to show that *gr* and *p* are not the same genes. Similarly, in these two families there is a segregation into 1 *Bu* : 1 *bu* (because of the elimination of $s^y$ male gametes), which might indicate that *gr* and *bu* were identical; but the first two families disprove that, since they are segregating for *gr* but not for *bu*. Families 3361 and 3373 support these suggestions, since they are both segregating for *gr* but for neither *p* nor *bu*. There is some evidence also to indicate that *sp* is not identical with *p* or with *bu*. Family 3101 is segregating into 1 *Sp* : 1 *sp* but has no buff plant, while in 3411 there are *bu* but no

*sp* plants, although the population is small (table 4). In families 3223, 3225, 3226, and 3411 there are pale-upper but no splotched plants, an indication that *sp* and *p* are not the same gene. There is no evidence regarding a possible connection between *sp* and *gr* and the yellow genes *y*, *gl*, and *ys*, since the first two were found only in orange families and the last three only in white.

With regard to linkage, there is probably no linkage between *gr* and the self-sterility alleles, since the *Gr* : *gr* ratios in the six families (3223, 3225, 3226, 3361, 3373, and 3411) that have a common self-sterility allele are not especially bad except for 3223 and the absence of *gr* plants in 3411. Since none of the families segregating for *sp* had a common self-sterility allele, nothing is known of possible linkage between *sp* and *s*.

Linkage with *y*, *gl*, and *ys* has not been tested, for *sp* and *gr* have not been found in *oo* plants. Linkage between *sp* or *gr* and *bu* or *p* cannot be tested, for it has not been possible to distinguish between *Sp* and *sp* and between *Gr* and *gr* in *cc* plants or between *Bu* and *bu* or *P* and *p* in *C* plants.

Since *c* is epistatic to *Gr* and *gr*, possible linkage between *gr* and *C* must be determined from the *Gr* : *gr* ratio in *C* plants in families which also throw recessive noncolored plants. The ratios in table 4 are generally sufficiently good so that there is no suggestion of linkage. The splotched gene, however, does appear to be linked with *C*, although the evidence is not so extensive as might be desired. Family 3004 arose from the cross *Cc Oo Spsp* × *Cc oo Spsp* and segregated into 20 colored orange nonsplotched : 2 colored orange splotched : 48 colored white : 10 noncolored orange : 15 noncolored white. The high percentage of whites in both the colored and noncolored classes is not readily explained and is not concerned in the immediate problem. The segregation of the colored, orange plants into 20 nonsplotched : 2 splotched, however, is interesting. On the basis of independent assortment, the deviation divided by the standard error is 1.72, but if *C* and *sp* are linked in the coupling phase in each parent, with 20% crossing over, the expected ratio for the twenty-two plants is 19.4 nonsplotched : 2.6 splotched, and the ratio of the deviation to the standard error is only 0.40. The observed ratio therefore agrees somewhat better with that expected on the basis of linkage. Families 3001 and 3103 also segregate for both *C* and *sp* and could be used to test for linkage, but since they are also segregating for granular, the problem also involved the epistasis of *sp* over *gr*. It has previously been shown that in families 3013 and 3101 (table 5) the observed numbers of the self-colored, splotched, and granular types show a closer fit to the expected ratio based on the epistasis of *sp* over *gr* than that based on the epistasis of *gr*. In families 3001 and 3103, using chi-square, the observed ratios were tested against the expected ratio based on (*a*) the epistasis of *sp* and linkage between *C* and *sp* with 20% crossing over, (*b*) the epistasis of *gr* and such linkage, (*c*) the epistasis of *sp* and no linkage, and (*d*) the epistasis of *gr* and no linkage. In both cases the observed ratio is in closer agreement with the first assumption. While such an agreement does not prove the assumption, at least it can be said that in four families the ratios are consistent in favoring the assumption that *sp* is epistatic, and in three families the ratios favor the view that *sp* and *C* are linked with about 20% crossing over.

### Red-outline (ro)

In the red-outline type, the anthocyanin pigmentation on the lower lip is generally confined to a thin red outline, although a few spots of red are also found near the tube, and on the splotched, red-outline type the splotched pattern may be present in addition to the outline. The upper lip is also red but the color is uneven (fig. 3). In some cases apparently the color is also greatly restricted in the upper lips, appearing only as a thin outline and a spot on each side of the lips near the tube. This gene apparently is expressed only in the presence of *C*.

Red-outline appears to be a recessive, although the data in regard to it are few. It is a difficult type to study because it is apparently affected by the presence of other genes. Unfortunately, the families in which it appeared were also segregating for a large number of genes which restrict the anthocyanin pigment to certain patterns, and in the course of following some of these other genes the red-outline type was neglected.

Family 3016 arose from a cross between two nonred-outline plants, 2901 (14) and 2901(16), and contained 11 nonred-outline and 6 red-outline. This looks like a simple recessive. Family 3005 was not so clear. The male parent was the same as in the previous family, but the female parent, 2901(7), appeared to be a red-outline phenotypically. Family 3005, however, consisted of 73 colored orange nonred-outline : 8 colored orange red-outline : 17 colored white nonred-outline : 2 colored white red-outline—all plants having anthocyanin pigment. The deficiency of red-outline cannot be explained. Family 3004 was from the cross 2901(7) × (13) and had 22 colored orange red-outline and 48 colored white nonred-outline. It is possible that both parents were recessive, although only the female appeared to be red-outline phenotypically, and that some modifying gene prevented the expression of red-outline in the parent which had a white background. Family 3009 was from 2901(10), a red-flowered plant phenotypically nonred-outline, and 2901(5), a noncolored orange-flowered plant. The ten colored offspring from this cross were all red-outline, although the one parent was not red-outline phenotypically. Some of the discrepancies in these families may arise from the difficulty of scoring red-outline when certain other pattern genes are present, but it is also possible that red-outline may be caused by two different genes that produce a somewhat similar effect. Red-outline will be considered tentatively as a simple recessive and given the symbol *ro*.

### Bicolor (bi)

The bicolor type is another modification of the *C* type and is apparently not expressed in *cc* plants. It has been traced through several generations in the line of plants with a white background. In the colored nonbicolor plants, the inner part of the corolla limb is Pomegranate Purple or a related color, but in the bicolor type the upper lip is Pomegranate Purple and the lower lip is Rose Color. The lower part of the tube is orange with deep purple dots in both the nonbicolor and bicolor plants. There is no evidence whether the bicolor gene can act in colored plants that have an orange background. In one of the earliest families, several plants were described and catalogued which appear as if they might have been bicolors on a colored orange background, but unfortunately they were found in a family that was not used to produce subsequent generations. No known bicolors appeared in the orange line that was continued for a number of

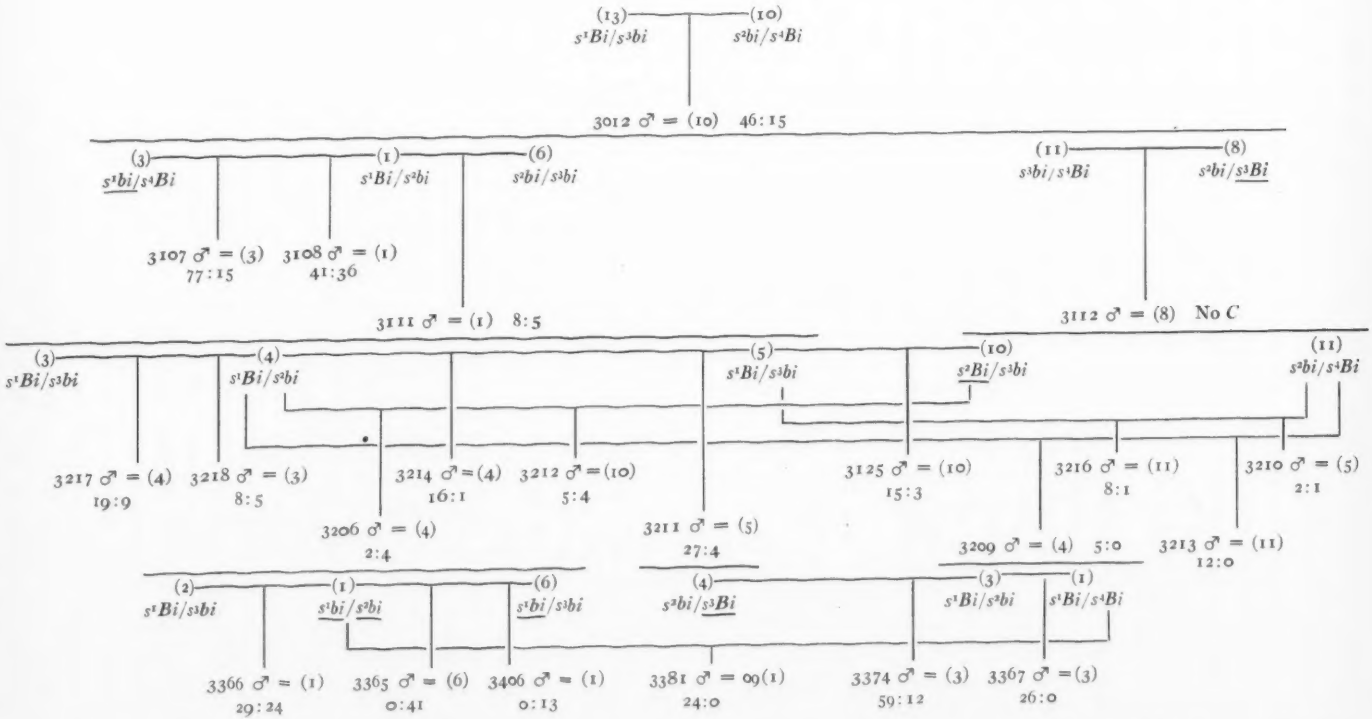



FIG. 4.—Pedigree diagram of families bearing on linkage between bicolor and self-sterility alleles. Ratios beneath pedigree numbers of families are ratios of non-bicolor to bicolor. Male parent of each family is indicated, as this is important when dealing with self-sterility. Crossover gametes indicated by a line beneath the *s* and *bi* genes.

generations. Crosses were made between bicolor plants on a white background and orange plants, but unfortunately the seeds of these crosses were no longer viable when they could be sown. This type has not been recovered in the new material as yet.

When two nonbicolor plants are crossed there are always nonbicolor plants in the offspring, although there are sometimes bicolors as well. Two families (3365 and 3406) arose from crosses between two bicolor plants. In one there were 41 bicolors and in the other there were 13, and in neither family was a nonbicolor found (fig. 4). A cross between a nonbicolor and a bicolor produced family 3381, which contained 24 nonbicolor and no bicolors. This last cross indicates that bicolor is recessive to nonbicolor.

Three families arose from a cross between two nonbicolors and contained both types of plants. In family 3012 there were 46 nonbicolors and 15 bicolors, while in families 3216 and 3210 the respective ratios of nonbicolor to bicolor were 8:1 and 2:1. In all three families and in the total of the three families the deviation from an expected 3:1 ratio was less than the standard error (table 6). One cross between a bicolor and a nonbicolor yielded 29 nonbicolored and 24 bicolored offspring (family 3366). On the basis of a 1:1 ratio the deviation in this family is 0.69 times the standard error. These families support the theory that bicolor is a simple recessive. The bicolor gene will be designated by the symbol *bi*.

Twelve other families arose from crosses between two nonbicolor plants and segregated into both nonbicolors and bicolors. As several showed a deviation more than twice as great as the standard error for a 3:1 ratio while some others had a deviation almost as large, it was decided to consider the possibility that the bicolor gene might be linked with the self-sterility alleles. This possibility was strengthened by the fact that the families with disturbed ratios were from parents which had a self-sterility allele in common. The self-sterility alleles of the various plants used as parents of these families and the self-sterility classes of the families themselves were known (**22**), so the first step in the problem was to fit the *Bi* and *bi* genes into a logical coupling or repulsion arrangement which would produce the aberrant ratios of these families. When these families were plotted in a diagram (fig. 4), it was seen that in some instances the *Bi* gene must have been coupled with the common self-sterility allele in the male, while in other families the male parent must have had the *bi* on the same chromosome as the common *s* allele. Seven families appeared to fall into the first category and five into the second, and these two groups are listed separately in table 6. Of the first seven families, only one deviated significantly, but the group as a whole segregated into a ratio of 126 nonbicolor to 64 bicolor, with a deviation 2.6 times the standard error for a 3:1 ratio. If there is linkage between *bi* and *s*, these seven families when combined would indicate a crossing over of 33.68%. For the five families in which *bi* in the male is coupled with the common *s* gene, the combined ratio is 158 nonbicolor to 34 bicolor and the deviation is again significant. If linkage is assumed, crossing over is 35.42%. When these two groups of plants are considered together, linkage comes to about 34.6%.

Individually, few of these families deviate significantly from a 3:1 ratio, but when the observed ratios are compared with the expected 3:1 ratios and with the expected ratios based upon

TABLE 6

RATIO OF NONBICOLOR TO BICOLOR IN THREE FAMILIES WHICH DO NOT HAVE COMMON SELF-STERILITY ALLELE AND IN TWELVE FAMILIES IN WHICH ONE ALLELE IS COMMON TO BOTH PARENTS

| Family | Genotypes of parents | Observed | | Expected (no linkage) | | | Expected (linkage with 34.6% crossing over) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Bi* | *bi* | *Bi* | *bi* | Dev. S.E. | *Bi* | *bi* | Dev. S.E. |
| 3012 | $s^1bi/s^3Bi \times s^2bi/s^4Bi$ | 46 | 15 | 45.75 | 15.25 | 0.07 | ..... | ..... | ..... |
| 3216 | $s^1Bi/s^2bi \times s^2bi/s^4Bi$ | 8 | 1 | 6.75 | 2.25 | 0.96 | ..... | ..... | ..... |
| 3210 | $s^2bi/s^1Bi \times s^1Bi/s^2bi$ | 2 | 1 | 2.25 | 0.75 | 0.33 | ..... | ..... | ..... |
| Total | ..... | 56 | 17 | 54.75 | 18.25 | 0.34 | ..... | ..... | ..... |
| 3108 | $s^1bi/s^2Bi \times s^1Bi/s^2bi$ | 41 | 36 | 57.75 | 19.25 | 4.41 | 51.82 | 25.18 | 2.63 |
| 3217 | $s^1Bi/s^2bi \times s^1Bi/s^2bi$ | 19 | 9 | 21.0 | 7.0 | 0.87 | 18.84 | 9.16 | 0.06 |
| 3218 | $s^1Bi/s^2bi \times s^1Bi/s^3bi$ | 8 | 5 | 9.75 | 3.25 | 1.13 | 8.75 | 4.25 | 0.44 |
| 3212 | $s^1Bi/s^2bi \times s^2Bi/s^3bi$ | 5 | 4 | 6.75 | 2.25 | 1.35 | 6.06 | 2.94 | 0.75 |
| 3211 | $s^1Bi/s^2bi \times s^1Bi/s^3bi$ | 27 | 4 | 23.25 | 7.75 | 1.56 | 20.86 | 10.14 | 2.35 |
| 3214 | $s^1Bi/s^3bi \times s^1Bi/s^2bi$ | 16 | 1 | 12.75 | 4.25 | 1.82 | 11.44 | 5.56 | 2.31 |
| 3375 | $s^2Bi/s^2bi \times s^2bi/s^2Bi$ | 10 | 5 | 11.25 | 3.75 | 0.74 | 10.10 | 4.90 | 0.05 |
| Total | ..... | 126 | 64 | 142.5 | 47.5 | 2.76 | 127.87 | 62.13 | 0.29 |
| 3107 | $s^1Bi/s^2bi \times s^1bi/s^4Bi$ | 77 | 15 | 69.0 | 23.0 | 1.93 | 76.08 | 15.92 | 0.25 |
| 3206 | $s^2Bi/s^3bi \times s^2Bi/s^3bi$ | 2 | 4 | 4.5 | 1.5 | 2.36 | 4.96 | 1.04 | 3.18 |
| 3215 | $s^1Bi/s^3bi \times s^2Bi/s^3bi$ | 15 | 3 | 13.5 | 4.5 | 0.82 | 14.89 | 3.11 | 0.06 |
| 3209 | $s^2bi/s^4Bi \times s^2Bi/s^2bi$ | 5 | 0 | 3.75 | 1.25 | 1.29 | 4.14 | 0.86 | 1.02 |
| 3374 | $s^2bi/s^3Bi \times s^1Bi/s^2bi$ | 59 | 12 | 53.25 | 17.75 | 1.57 | 58.72 | 12.28 | 0.09 |
| Total | ..... | 158 | 34 | 144.0 | 48.0 | 2.33 | 158.78 | 33.22 | 0.15 |

linkage between *bi* and *s* with 34.6% crossing over, it is found that the agreement with the latter ratio is better in nine of the twelve families. In two families (3211 and 3214) the observed ratio agrees better with the expected 3:1 ratio. The reasons for this are not clear, but these families are from reciprocal crosses and some other factor may be operating. The third family (3206) contains only six *C* plants, deviates significantly from both expected ratios, and probably should not be included.

Only one family resulted from a cross between a heterozygote and a bicolor. This was family 3111, whose parents were 3012(6) and 3012(1). Family 3012 arose from two of the original plants and segregated into a perfect 3:1 ratio. The parents had no self-sterility alleles in common, for four self-sterility classes were found in 3012. Plant 3012(6) was $s^2s^3$ and a bicolor. Plant 3012(1) was $s^1s^2$ and heterozygous. When this plant was crossed as a male on to 3012(3) to produce family 3108, it appeared that the *Bi* gene must be coupled with the $s^1$ gene as the ratio in 3108 could not otherwise be explained. If plant 3102(1) is $s^1Bi/s^2bi$, the $s^2$ gametes should be eliminated in the cross 3012(6) × (1), and there should be an excess of *Bi* plants over the expected 1:1 ratio, all bicolors appearing merely as the result of crossing over. Only thirteen *C* plants were raised in this family, and eight were nonbicolor and five were bicolor. On the basis of no linkage the deviation is 0.83 times the standard error, but on the basis of linkage it is only 0.29 times the standard error. While neither ratio deviates significantly, again there is better agreement with the theory based on linkage.

Even though a series of disturbed ratios fits into a scheme based on linkage with self-sterility alleles, the possibility that the morphological genes are eliminated for some other reason must also be considered. Linkage with *Ga* genes, such as MANGELSDORF and JONES (**16**) found in maize, with complete elimination of the *ga* gametes and 34.6% crossing over between *Ga* and *bi*, could also explain some of these disturbed ratios. To determine whether linkage with such genes is the cause of the gametic elimination of *Bi* or *bi* genes, the ratio of the two self-sterility classes in both the *Bi* and *bi* phenotypes should be examined. EAST and MANGELSDORF (**7**) showed that when the two parents have a common self-sterility allele, the self-sterility class of the mother is always absent in the progeny, and that, of the two classes in the offspring, one is identical with that of the paternal parent while the other is different from the classes of both parents. If *Ga* genes were operating, the ratio of *Bi* to *bi* would be determined by the nature of the coupling of *Ga* and *bi*. Half the *Bi* plants would belong to the paternal self-sterility class and half to the nonparental class, while half the *bi* plants would be of the paternal class and the other half would be nonparental. If *bi* was linked with the *s* genes, the ratio of paternal to nonparental classes would not be 1:1 for both the bicolor and nonbicolor types, but it would be different for each. For example, if *Bi* in the male were coupled with the common self-sterility allele, the paternal *Bi* plants would be more numerous than the nonparental *Bi* type, while more nonparental *bi* plants than paternal *bi*'s would be expected. Likewise, if *bi* were coupled with the common allele in the male, the nonparental nonbicolors should exceed the paternal, while there should be more paternal than nonparental bicolors. Table 7 lists the total of these four groups for three families in which *Bi* was coupled in

the male and for four families in which *bi* was coupled in the male. While chi-square is not significant for either theory, it is smaller in each case when the observed ratio is tested against the expected, based on linkage of *bi* and *s*, than when tested against a possible linkage of *bi* with *Ga* genes and absence of linkage between *bi* and *s*. This test affords additional evidence to suggest that the gene for bicolor is linked with the self-sterility alleles.

These families do not segregate for *C* and therefore contain all colored plants. Since there are no white plants, the ratio of nonbicolor to bicolor should be 3:1 if the disturbed ratios in segregating families are due to linkage between *bi* and *C* rather than between *bi* and *s*. These two nonsegregating families support the theory that *bi* is not linked with *c*. This idea is further supported by family 3012, which is segregating into colored and noncolored plants but in which the ratio of nonbicolor to bicolor is a perfect 3:1 ratio.

TABLE 7

COMBINED RATIOS OF NONBICOLOR TO BICOLOR AND OF PATERNAL TO NONPARENTAL SELF-STERILITY CLASSES. FOLLOWING THE OBSERVED RATIOS ARE (1) EXPECTED RATIOS IF THE GAMETIC ELIMINATION OF *Bi* AND *bi* IS NOT THE RESULT OF LINKAGE BETWEEN BICOLOR AND SELF-STERILITY ALLELES AND (2) EXPECTED RATIOS IF *bi* AND *s* ARE LINKED WITH 34.6% CROSSING OVER

| Linkage in male parent | No. of families | Observed | | | | Expected (1) | | | | $X^2$ | Expected (2) | | | | $X^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Non-bicolor | | Bicolor | | Non-bicolor | | Bicolor | | | Non-bicolor | | Bicolor | | |
| | | P* | N | P | N | P | N | P | N | | P | N | P | N | |
| *Bi* coupled | 3 | 12 | 9 | 2 | 5 | 9.4 | 9.4 | 4.6 | 4.6 | 2.24 | 10.8 | 8.0 | 3.2 | 6.0 | 0.87 |
| *bi* coupled | 4 | 14 | 17 | 7 | 2 | 16.5 | 16.5 | 3.5 | 3.5 | 4.54 | 15.4 | 17.6 | 4.6 | 2.4 | 1.47 |

* P, paternal; N, nonparental.

As in the case of the buff gene (**25**), since *bi* cannot be tested in *cc* plants, and since linkage between *bi* and *C* would therefore give disturbed ratios of *Bi* to *bi* in *C* plants of families segregating for *C*, the possibility that the disturbed ratios in these families are due to linkage between *bi* and *C* rather than between *bi* and *s* must be considered. If linkage were between *bi* and *C*, the ratios should not be disturbed in families which do not segregate for *C*. In families 3107 and 3108 (table 6) the ratios are very much nearer those based on linkage between *bi* and *s* than those based on no such linkage. In fact, in 3107 the deviation from the expected 3:1 ratio is highly significant while in 3108 it is almost significant.

Since *bi* appears to be linked with *s*, it should also be linked with buff, as the latter is closely linked with *s*. This possible linkage cannot be tested directly, however, since *bu* is expressed only in *cc* plants, while *bi* is expressed only in plants containing the dominant allele *C*. For a similar reason, it is not possible to test linkage between bicolor and pale-upper, but since *bi* is linked with *s* while *p* is not, *bi* and *p* cannot be linked. For a similar reason, *bi* cannot be linked with *O* and is probably not linked with *y*, *gl*, *ys*, or *gr*. It is not known whether *sp* and *s* are linked. No data are available to test linkage between *bi* and *sp*, since the

bicolor plants were found only in the *oo* strain while the splotched type was identified only in *O* plants.

### Chimaeras

Chimaeras throw some light on the recessive nature of noncolored, just as they did for nonorange and for yellow (25). Several have been found. Three plants were scored as Carmine and were in families in which at least some of the plants were heterozygous for *C*. In each plant there was an orange streak in the lower lip. One other plant from one of these same families had several orange streaks in both lips. In one plant of a family segregating for *C* on a white background, there was a white streak on the upper lip which was otherwise Pomegranate Purple. In one flower of a plant of family 3372, a more complicated chimaera was found. This plant was a carmine-flowered plant on an orange background. The color was uneven, but as the family was composed of several different pattern types it was not certain whether the irregularity in color was due to *sp*, to some other pattern gene, or to both. It had one wide chimaera in the center of the lower lip. This streak was Pomegranate Purple, but on each side of it there was a narrow streak of white. Apparently there was considerable mitotic disturbance when the flower was developing, resulting in the loss of the *O* gene at one point and the loss of *C* at two other points within the area that had lost the *O*.

The presence of orange streaks in red flowers and white streaks in purple flowers supports the idea that in the absence of some confusing, modifying genes, *CO* is red and *Co* is purple. Two plants from a family homozygous for *O* were Ox-blood Red but had one flower with several white chimaeras on the upper lip. At first sight these appear to be contradictory to the general rule, but a close examination showed that within the white sectors the entire epidermis was missing, so that the white streaks represented subepidermal tissue.

### Summary

1. Colored flowers are dominant over white and are the result of gene *C*, a gene for anthocyanin pigmentation. In general, this gene in combination with *O* produces Carmine or Ox-blood Red flowers, while *C-oo* plants are usually Pomegranate Purple, Amaranth Purple, or Rose Color. Certain modifying genes vary the color, and in some plants it is impossible to determine from the color of the anthocyanin whether the background is white or orange. Gene *C* does not appear to be linked with the self-sterility alleles, or with *O*, *bu*, *p*, *y*, *gl*, or *ys*.

2. Two of the important modifiers of *C* are the splotched (*sp*) and granular (*gr*) genes. Both are recessives and are expressed only in *C* plants. In the splotched type the color is uneven and frequently found in irregular blotches and streaks. This gene is variable in its expression. In granular, there are numerous small colorless dots in the anthocyanin region, and again the expression of the gene is not uniform. Apparently neither *sp* nor *gr* is merely an expression of *bu*, *p*, *y*, *gl*, and *ys* on a *C* background. Several families indicate that *sp* is epistatic to *gr* and that *sp* and *C* are linked with about 20% crossing over.

3. Red-outline (*ro*) is another modification of *C*. The pigmentation tends to be restricted to an outline on the margin of the lower lip or of both lips, although other areas of color are usually present also on the lips. It appears to be a simple

recessive, but the data are meager and some ratios are poor. Apparently it is hypostatic to *c*.

4. Bicolor (*bi*) is a modification of *C* in which the anthocyanin color is considerably paler on the lower lip. This has appeared with certainty only in the strain homozygous for *o*, but there is some indication that it also affects *CO* plants. In general, the upper lip is Pomegranate Purple and the lower lip Rose Color. It does not seem to have any expression in *cc* plants. It is linked with the self-sterility alleles, with about 34.6% crossing over.

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# INTERACTION OF NITROGEN NUTRITION AND PHOTOPERIOD AS EXPRESSED IN BULBING AND FLOWER-STALK DEVELOPMENT OF ONION

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## Introduction

Photoperiod has been shown (**2**, **3**, **5**, **6**) to exercise strong control over bulb formation in onion. Long photoperiods promote bulbing, and short ones delay, or—if sufficiently short—completely inhibit it. The level of nitrogen nutrition has also been found (**1**, **9**) to influence bulb formation, high levels delaying bulbing and low levels accelerating it. The effects of these two factors on the flowering of onion have received much less attention, but knowledge of such effects would be helpful to onion breeding and to an understanding of the photoperiodic reactions involved. Previous studies have not simultaneously varied the nitrogen nutrition and the photoperiod, so such experiments have been undertaken here in order to determine whether any interactions exist, and, if so, what their effect is on bulbing and flowering.

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## Material and methods

These experiments were conducted in the greenhouse at various times of the year. The first experiment reported did not deal with different levels of nitrogen nutrition, and the plants were initially grown in soil in 4-inch pots. Later they were transplanted to soil benches. The plants for the other two experiments were grown in sand in 1½-liter glazed crocks. In these tests the concentration of available nitrogen was varied.

In the nutrition experiments, eight solutions (table 1) were used. Solutions 1, 2, 7, and 8 were employed in experiment II and solutions 3–7, inclusive, in

experiment III. Solution no. 1 differed from all others in both the form and the concentration of the major elements, but solutions 2–8 were identical in composition, except for their concentrations of nitrogen. All solutions were complete except no. 2, which contained no nitrogen. A modified Hoagland solution (4) was employed to supply the minor elements in each nutrient solution. The solutions were applied daily in sufficient quantity to insure adequate flushing from the crock drains.

## Results

### Experiment I

The plants for this experiment were produced from hybrid onion bulbs[4] ($F_2$ population of male-sterile Italian Red 13–53 × Red Wethersfield) planted in soil in pots October 29, 1941. They were grown until December 19 in controlled environment rooms,[5] where they received 12-hour photoperiods and four different combinations of night and day temperature. Following this, they were moved to the greenhouse, transplanted to soil benches, and subjected to three different lengths of photoperiod until April 10, when the experiment was terminated.

TABLE 1

Composition of nutrient solutions

| Solution no. | Molar concentration of c.p. salts | | | | | | N (p.p.m.) |
|---|---|---|---|---|---|---|---|
| | $MgSO_4 \cdot 7H_2O$ | $CaCl_2 \cdot 2H_2O$ | $KH_2PO_4$ | $(NH_4)_2SO_4$ | $Ca(NO_3)_2 \cdot 4H_2O$ | $NaNO_3$ | |
| 1 | 0.0023 | ..... | 0.0023 | 0.0007 | 0.0045 | ..... | 73 |
| 2 | .0058 | 0.0030 | .0015 | ..... | ..... | ..... | 0 |
| 3 | .0058 | .0030 | .0015 | ..... | ..... | 0.0002 | 3 |
| 4 | .0058 | .0030 | .0015 | ..... | ..... | .0009 | 13 |
| 5 | .0058 | .0030 | .0015 | ..... | ..... | .0018 | 26 |
| 6 | .0058 | .0030 | .0015 | ..... | ..... | .0028 | 39 |
| 7 | .0058 | .0030 | .0015 | ..... | ..... | .0037 | 52 |
| 8 | 0.0058 | 0.0030 | 0.0015 | ..... | ..... | 0.0075 | 105 |

Photoperiods both longer and shorter than those occurring naturally were employed. Supplemental light from 100-watt incandescent filament bulbs was used to extend the natural photoperiods. In experiments I and II the intensity of supplemental light was 40–50 foot-candles and in experiment III about 25 foot-candles at pot level.

The plants requiring photoperiods shorter than those occurring naturally were either covered with a double thickness of black sateen cloth or moved into dark chambers after they had received the desired duration of light. The data were subjected to variance analysis (8) and the significance of differences determined by the *t*-test.

The four temperature treatments applied while the plants were in the control rooms consisted of 65° F. night and day, 45° F. night and day, and both night and day combinations of these two temperatures. The photoperiods employed after the plants were moved to the greenhouse

[4] All onion plant material was supplied by Dr. Henry Jones, Bureau of Plant Industry Station, Beltsville, Maryland.

[5] A complete discussion of the features of these rooms has recently been reported by Parker (7). The light is secured from a combined carbon-arc Mazda source and is of a quality similar to sunlight.

were 10, 14, and 18 hours. Each photoperiodic treatment included plants from all the four temperature treatments, and the experiment was set up in duplicate in adjoining greenhouse sections.

Frequent dissections were made of plants from the various treatments, beginning December 19 when they were moved to the greenhouse, but the first inflorescence primordia were not observed until January 16. Flower stalks first became macroscopically visible by the middle of February, and by April 10

TABLE 2

INFLUENCE OF TEMPERATURE AND PHOTOPERIOD ON PRODUCTION OF FLOWER STALKS BY HYBRID ONION PLANTS. SIXTEEN PLANTS PER LOT

| TEMPERATURE (° F.) | | NUMBER OF FLOWER STALKS DEVELOPED ON PHOTOPERIODS | | |
|---|---|---|---|---|
| *Light period* | *Dark period* | 10-hour | 14-hour | 18-hour |
| 65 | 65 | 4 | 10 | 7 |
| 65 | 45 | 4 | 12 | 11 |
| 45 | 65 | 2 | 11 | 14 |
| 45 | 45 | 8 | 8 | 12 |

(when the experiment was terminated) they were present on 103 of the 192 plants. The remaining eighty-nine plants were dissected at that time, and only three contained inflorescence primordia.

The temperature variables to which the plants were subjected during the first weeks of growth did not result in any statistically significant flowering differences (table 2), but the plants that had received no low temperature during their early development produced fewer flower stalks than did those of the other three treatments. The greatest differences in flowering were associated with differences in photoperiod. About equal numbers of plants on 14- and 18-hour photoperiods produced flowers, but less than half as many flowered on 10-hour photoperiod, and at the time the experiment was terminated these were in a less advanced stage of development than those of the longer photoperiods. On many of the 10-hour plants the bract that normally envelops the inflorescence grew into a long, curved, green structure resembling a vegetative leaf blade. The 14-hour plants exhibited the same character to less degree, but it was not observed in the 18-hour lots. Representative plants from the 10-, 14-, and 18-hour photoperiods are shown in figure 1.

### EXPERIMENT II

On July 21, 1942, two closely related plant lines, 74 and 75, were planted outdoors in soil in thumb-pots. These lines had been derived from Yellow Bermuda × White Persian $F_1$'s, backcrossed once to Yellow Bermuda and then selfed. The germinated seedlings were subjected to 8-hour photoperiods for 6 weeks and then were washed free of soil and transplanted to quartz sand in glazed crocks in the greenhouse. During the following 2 weeks, while still on 8-hour photoperiods, the plants were given a complete nutrient solution (solution 7; table 1) supplying 52 p.p.m. of nitrogen. Experimental treatments involving two lengths of photoperiod and four nutrient solutions differing mainly in their nitrogen content were begun at the close of this period, on September 28.

Eight treatment blocks were established, four each for the 11- and the 13-hour photoperiods. Each block consisted of four rows of ten plants. One continuous half of each row contained plants of line 74 and the other half plants of line 75, the relative position of the plant lines alternating from row to row.

Four nutrient solutions (1, 2, 7, and

8; table 1) were used in each block, a different one for each row. Solution 1 supplied nitrogen in the form of both nitrate and ammonia at a combined rate of 73 p.p.m., while solutions 2, 7, and 8—which contained no ammonia—supplied nitrate nitrogen at rates of 0, 52, and 105 p.p.m., respectively.

These treatments were continued until November 2, 1942, at which time all plants that had received the minus-nitrogen solution had begun to bulb on both photoperiods, with the most advanced bulbing on the longer photoperiod. Bulbing was not evident in any other treatment on either photoperiod. At this time all plants of both photoperiods that had been receiving 105 p.p.m. of nitrogen were placed on minus nitrogen, and those that had been receiving minus nitrogen were placed on 105 p.p.m. of nitrogen. By January 1, 1943, the plants that had been shifted from minus to high nitrogen no longer showed any evidence of bulbing; however, the lots that had been shifted from high to minus nitrogen were in an advanced stage of bulb development. This reciprocal transfer demonstrated that a high level of nitrogen would suppress bulb formation even after the process was initiated, and that withholding nitrogen would promote bulbing even in plants that had previously been grown on a high nitrogen level.

By January 14, all plants except those on the highest nitrogen level had begun to bulb, but none had approached maturity. At this time the bulb diameter of each plant was recorded, and the means for these measurements are shown in table 3. Since none of the bulbs were mature, these diameters indicate only the relative stages of development attained at the time the measurements were made. The plants maintained on minus nitrogen were characterized by erect, nonsucculent, pale green leaves; those supplied with 105 p.p.m. of nitrogen had declining, succulent, dark green leaves. Those of the other two solutions were intermediate in these characteristics and also in their degree of bulbing.

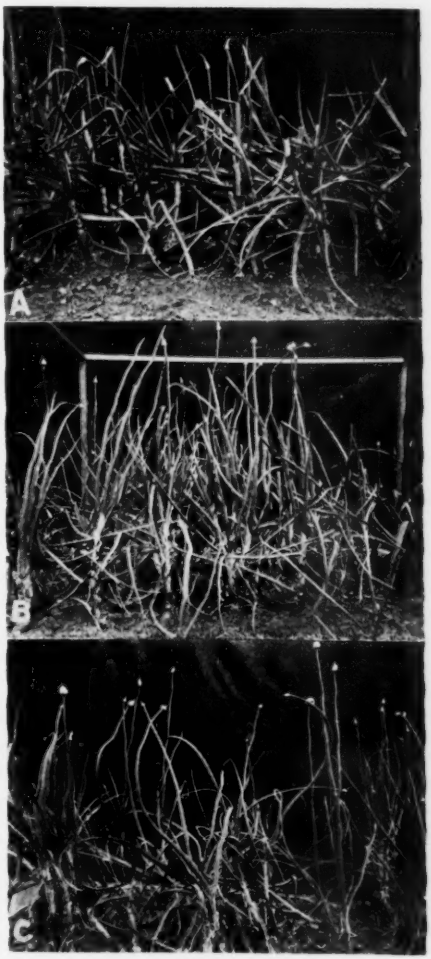


FIG. 1.—*Hybrid onion plants grown on photoperiods of A, 10 hours; B, 14 hours; C, 18 hours. Photographed April 3, 1942.*

Differences in bulb diameter signifi-

cant at the 1% level were associated with photoperiod, solution, and replication, but not with plant line. The F-values of photoperiod and solution were each more than twice the F-value of replication. There was significance at the 5% level for the interaction of photoperiod and solution but not for any of the remaining two-factor interactions. This is interpreted to mean that the progressive increase in difference between the two photoperiods that occurred as the nitrogen content of the solution was decreased was significant.

TABLE 3

INFLUENCE OF PHOTOPERIOD AND NITROGEN NUTRITION ON BULBING OF ONIONS FROM SISTER PLANT LINES. TWENTY PLANTS PER LOT

| PHOTOPERIOD (HOURS) | PLANT LINE | MEAN DIAMETERS (CM.) OF BULBS GROWN IN VARIED NITROGEN CONCENTRATIONS* | | | |
|---|---|---|---|---|---|
| | | 105 p.p.m. | 73 p.p.m. | 52 p.p.m. | 0 p.p.m. |
| 13..... | 75 | 1.6 | 2.6 | 2.8 | 3.6 |
| | 74 | 1.7 | 2.8 | 2.5 | 3.2 |
| 11..... | 75 | 1.4 | 2.1 | 1.9 | 2.5 |
| | 74 | 1.4 | 2.0 | 1.9 | 2.6 |

* Diameter difference of 0.6 cm. required for significance at 1% level.

Under both daylengths (table 3) the greatest degree of bulbing was associated with the minus-nitrogen level, while the least degree was associated with the highest level. Bulbing was intermediate in the other two treatments, and—although part of the nitrogen was supplied in the form of ammonia—for one of these treatments there was no significant difference in the stages of bulb development under the two treatments. With each nitrogen treatment the degree of bulbing was greatest under the longer daylength.

The most outstanding effect of the nitrogen factor is evident (table 3) in the degrees of bulbing on the 11-hour photoperiod as against those on the 13-hour. Plants receiving a minus-nitrogen solution and an 11-hour photoperiod exhibited a greater degree of bulbing than did those receiving the 105 p.p.m. nitrogen solution and 13-hour photoperiod. Statistically, this difference is highly significant.

The data in table 3 indicate no apparent difference between the bulbing of the two plant lines under comparable experimental treatments. This behavior was to be expected, since the parental stocks from which these two lines came had been selected for uniformity with respect to bulbing performance.

Single flower stalks were visible on each of three plants in the experimental population when the bulb diameters were recorded on January 14, so the experiment was continued until March 27, to demonstrate any effects of the various treatments upon flowering. No change was made in the nutrient treatments during this time but one hour was added to each photoperiod, making them 12 and 14 hours each. During the 10-week period, daily records were taken of emergence of flower stalks of individual plants.

The most obvious result of this part of the experiment was the difference in flowering response of the two lines when subjected to the shorter photoperiod (table 4). All but one of the eighty plants of line 75 flowered, but only twenty-three of line 74 flowered under this condition. The remaining fifty-seven plants of line 74 were not dissected; it is possible that the inflorescence primordia may have been present in some of them. This difference in flowering response between the two lines with the shorter pho-

toperiod is great enough to be highly significant.

A similar but less obvious difference appeared between the two lines on the longer photoperiod. Ten plants of line 75 failed to flower but all of these had formed mature bulbs. In line 74 fourteen plants failed to flower but only six of these had formed mature bulbs. Thus, all the seventy non-bulbing plants of line 75 flowered, but eight of the seventy-four non-bulbing ones in line 74 failed to flower. This difference between the two lines may not be statistically significant but it is in the same direction as the greater difference found on the shorter photoperiod.

TABLE 4

INFLUENCE OF PHOTOPERIOD AND NITROGEN NUTRITION ON PRODUCTION OF FLOWER STALKS OF ONIONS FROM SISTER PLANT LINES. TWENTY PLANTS PER LOT

| PHOTOPERIOD (HOURS) | | PLANT LINE | NO. FLOWER STALKS PRODUCED BY PLANTS GROWN IN VARIED NITROGEN CONCENTRATIONS | | | |
|---|---|---|---|---|---|---|
| Sept. 28–Jan. 14 | Jan. 14–Mar. 27 | | 105 p.p.m. | 73 p.p.m. | 52 p.p.m. | 0 p.p.m. |
| 13..... | 14 | 75 | 19 | 16 | 19 | 16 |
| | | 74 | 16 | 18 | 18 | 14 |
| 11..... | 12 | 75 | 19 | 20 | 20 | 20 |
| | | 74 | 3 | 4 | 9 | 7 |

Although there was no significant difference in number of plants of line 75 flowering under the two photoperiods, there was considerable difference in the degree of flower-stalk development. The inflorescences of plants in the lot on the longer photoperiod were considerably more advanced than those on the shorter photoperiod. Figure 2, showing representative long-day and short-day treatment blocks, illustrates this difference in degree of development of flower stalks and umbels. Figure 3 shows individual plants representative of the types developed in certain of the treatments at the close of the experiment.

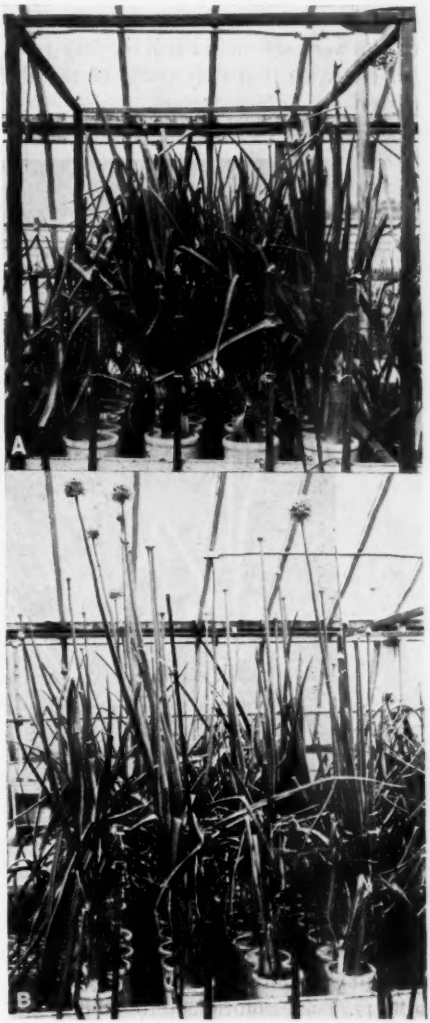


FIG. 2.—Onion plants of sister lines grown on photoperiods of *A*, 12 hours; *B*, 14 hours. Photographed March 13, 1943.

### EXPERIMENT III

Uniform sets, $\frac{3}{8}$–$\frac{1}{2}$ inches in diameter, of Ebenezer onion were planted in quartz sand in crocks in the greenhouse. The crocks were set on six trucks, fifty pots per truck, so that they could be readily moved to adjacent dark rooms. Ten plants on each truck received one of five nutrient solutions daily (solutions 3–7; table 1). These solutions differed only in concentration of nitrogen. Although the relative balance of major-salt ions was not constant for all five solutions, the only plant symptoms evident during the experiment occurred in the low-nitrogen lots and could be ascribed to a deficiency of nitrogen. Photoperiods of 10, 12, and 14 hours were maintained on pairs of trucks throughout the experiment. The daily duration of natural light was the same for all lots and varied from $8\frac{1}{2}$ to 10 hours as the season progressed. This period of natural light was extended by incandescent light of approximately 25 foot-candles to give the desired photoperiod.

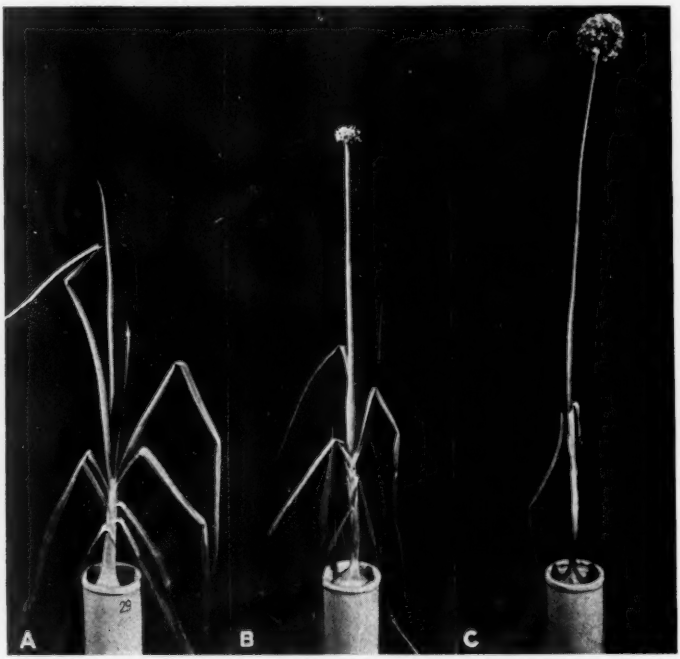


FIG. 3.—Onion plants grown with different photoperiods and with different concentrations of nitrogen: *A*, 12 hours and 52 p.p.m. nitrogen; *B*, 12 hours and 0 p.p.m. nitrogen; *C*, 14 hours and 0 p.p.m. nitrogen. Photographed March 13, 1943.

The treatments were started January 7, 1943, when the sets were planted, and the first leaf blade had emerged from all sets by January 16. The plants were grown on the several photoperiods and nitrogen treatments for 8 weeks, ending March 17. At this time some of the plants on the 14-hour photoperiods had devel-

oped bulbs that were nearly mature, so the experiment was terminated. The roots were discarded and the rest of the plant was divided into leaf-base and leaf-top portions. The leaf-base part of some of the plants was a definite bulb but in others varied from a slightly swollen to an unswollen pseudostem. In the former case the leaf tops were cut just above the bulb and in the latter they were cut 1 inch above the basal plate. The tissue was dried in a forced-draft oven at 80° C. for 48 hours and the dry weight of each fraction determined.

The dry weight of bulb or pseudostem expressed as percentage of the total dry weight of the plant was used as a measure of the degree of bulbing. The mean values of these percentages, as well as the mean dry weight per plant for the various treatments, are given in table 5.

All the plants on 14-hour photoperiod bulbed, regardless of the level of nitrogen supplied, and those from the four solutions of highest nitrogen composition attained about the same average weight. Although the bulbs from the solution of 3 p.p.m. of nitrogen were little more than half as large as the rest, they reached maturity at approximately the same time. The bulbs of all lots contained about the same percentage of the weight of the whole plant.

While all plants bulbed on the 14-hour photoperiods, only twelve of the 100 plants on 12-hour photoperiod showed marked bulbing. Five of the latter were grown with 3 p.p.m. of nitrogen and the remaining seven with 13 p.p.m. In the 10-hour treatments there were no well-developed bulbs, but in some lots the plants had begun to swell slightly at the base. The data on degree of bulbing for plants of the 10- and 12-hour lots (table 5) indicate only the relative development attained at the time of harvest.

Bulbing differences between plants of the 14-hour and those of either the 12- or the 10-hour lots were obviously significant. Accordingly, an analysis of variance was limited to the data on degree of bulbing of the 12-hour and 10-hour lots. Such data show significance at the 1% level for nitrogen concentration but not for photoperiod or replication. There is no significance shown for any two-factor interaction.

TABLE 5

EFFECT OF PHOTOPERIOD AND NITROGEN SUPPLY ON DRY WEIGHT OF ONION PLANTS AND DEGREE OF BULBING.* DATA BASED ON MEAN OF TWENTY PLANTS PER LOT

| Concentration of nitrogen (p.p.m.) | Dry weight (gm.) of plants grown on photoperiods | | | Degree of bulbing on photoperiods | | |
|---|---|---|---|---|---|---|
| | 10-hour | 12-hour | 14-hour | 10-hour† | 12-hour† | 14-hour |
| 52..... | 2.7 | 2.8 | 1.8 | 12.5 | 13.4 | 68.8 |
| 39..... | 2.5 | 1.8 | 2.0 | 16.9 | 15.2 | 66.0 |
| 26..... | 1.9 | 1.9 | 1.7 | 17.0 | 16.6 | 64.5 |
| 13..... | 1.8 | 2.0 | 1.9 | 17.0 | 18.9 | 66.0 |
| 3..... | 0.7 | 0.7 | 1.0 | 22.9 | 22.8 | 69.0 |

* Degree of bulbing = $\frac{\text{Dry weight of bulb or pseudostem}}{\text{Dry weight of total plant}} \times 100$.

† Difference of 5.9 required by the nitrogen treatments for significance at 1% level.

## Discussion

GARNER and ALLARD (2), MCCLELLAND (6), WILSON (9), and MAGRUDER and ALLARD (5) have indicated the dependency of onion bulbing upon the relative length of day. In all varieties investigated they report that bulbing occurred under the longer photoperiods and was inhibited or delayed by the shorter ones. WILSON, one of the first to study the effects of nutrition on the bulbing of onion, investigated the relation of nitrate nitrogen to the carbohydrate and nitrogen content of onion plants grown

either in sand culture or soil. He noted that excessive quantities of nitrate tended to depress total yields of bulbs without affecting the yield of tops. Since photoperiod was not a variable in this particular experiment, WILSON was unable to observe any interactive effects of nitrogen nutrition and photoperiod.

BREMER (1) was the first to note that earlier bulbing of onion could be obtained through a nitrogen deficiency. He completed both greenhouse and field experiments, but like WILSON, he used but one photoperiod in any one experiment. The development attained at harvest time was indicated by expressing the weight of bulbs as a percentage of the total plant weight. On this basis, BREMER reported earlier bulbing if the plants received a deficient level of nitrogen—either in soil or in sand culture.

The data presented in this paper substantiate the fact that length of photoperiod is a controlling factor in onion bulbing. Plants grown with the duration of photoperiod well above a certain critical range bulb immediately, and those grown below do not. This critical range may vary somewhat for different varieties, but within this range the process of bulb formation is slower than it is with longer photoperiods, and the plants are more susceptible to the influence of other environmental factors. This is illustrated by the data of experiments II and III. The plants in experiment II were grown near the critical range of photoperiod for bulbing, and nitrogen nutrition affected the degree of bulbing. In experiment III the longest photoperiod was farther from the critical range, and nitrogen nutrition did not influence the bulbing response. Thus, an interaction of photoperiod and nitrogen nutrition on bulbing was evident when the plants were grown near the critical photoperiod. Under these conditions a deficient level of nitrogen has the same effect as lengthening the photoperiod, and a high nitrogen supply has the same effect as shortening the photoperiod.

It is well recognized that varieties of onions differ in bolting habit. Experiment II shows that photoperiod may be one of the controlling factors in the flowering response of onions. One of the two strains of experiment II showed rather striking differences in flowering between the two photoperiods employed, while the other showed only slight differences. Low temperature during the development of the plant is known to be a most important factor in determining whether onions will flower, but these data indicate that photoperiod may also play a prominent role.

The difference in flowering response on short photoperiod of these two closely related strains of onion was not unexpected. The two strains were derived from parent stocks selected for uniformity of bulbing; no particular attention had been given to selection for uniformity of bolting. By using controlled photoperiods such selections could be readily made, if desired.

Plant breeders desirous of producing successive generations in the shortest time possible can grow their onions from seed to seed without an intervening dormant period if the environment is correctly manipulated. If this is to be done, the seedling should be started and maintained on a photoperiod below the critical for bulbing. After the plants have grown for several months with relatively low temperatures, the photoperiod can be lengthened to promote flowering but must not be lengthened so much that the plants will produce mature bulbs without flowering. High concentrations of nitrogen added at this time help to de-

press the bulbing tendency resulting from the longer photoperiod. The data of this study do not indicate that such nitrogen applications interfere with floral development.

## Summary

1. A study was made of the several effects of photoperiod and nitrogen nutrition upon the bulb and flower-stalk development of onion. Plants were grown from seed, sets, and mature bulbs, and with soil and in sand culture.

2. Plants grown on photoperiods substantially longer than that critical for bulb formation showed no difference in bulb development when supplied different amounts of nitrogen.

3. Plants grown on a photoperiod at or near that critical for bulb formation exhibited the greatest bulb development with the lowest nitrogen concentration and the least with the highest concentration.

4. Under certain environmental conditions nitrogen and photoperiod can interact to influence bulb development of certain onion varieties.

5. Onion plants of sister lines gave similar bulbing response on photoperiods of identical duration. However, the two lines differed in their production and development of visible floral stalks, especially on the shorter of the photoperiods. Development of floral stalks was favored by long photoperiods.

6. In a hybrid line unrelated to the sister lines, long photoperiods also favored flower-stalk development, but low temperatures applied during the early growth of these plants were without measurable effect.

7. Onion plants were grown from seed to seed without an intervening dormant bulb stage by controlling the photoperiod and concentration of nitrogen available for growth.

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# HISTOLOGICAL CHANGES IN BINDWEED AND SOW THISTLE FOLLOWING APPLICATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID IN HERBICIDAL CONCENTRATIONS[1, 2]

H. B. TUKEY, C. L. HAMNER, AND BARBARA IMHOFE

## Introduction

Recent investigations have called attention to a new principle in herbicidal action following application of 2,4-dichlorophenoxyacetic acid to bindweed, sow thistle, and other plants (**4, 5, 9**). Most other herbicides, such as sodium chlorate and sulphuric acid, depend upon caustic properties to destroy the parts of the plant with which they come into immediate contact. The action of 2,4-dichlorophenoxyacetic acid, however, is physiological (**11**); the chemical, or its effect, travels some distance through the plant to induce a telemorphic response (**2**) which contributes to the death of the plant. The typical gross symptoms following application to bindweed and sow thistle are curvature and chlorosis of above-ground parts and proliferations of below-ground parts. The external evidence is an indication of changes taking place within the plant. This paper presents a study of these responses on a cellular level, to determine, if possible, how they contribute to the death of the plant.

## Material and methods

The plants used for anatomical and histological study were bindweed (*Convolvulus arvensis* L.) and sow thistle (*Sonchus arvensis* L.). They were growing in a cultivated area in the immediate vicinity of Geneva, New York, the summer of 1944. The soil of the area is characteristically heavy, has a pH of 6.5, and is provided with a fairly uniform moisture supply.

The plants were sprayed with an aqueous solution of 2,4-dichlorophenoxyacetic acid at 1000 p.p.m. in a carrier of 0.5% Carbowax 1500, using a knapsack sprayer. Bindweed was treated on July 14 and 24, 1944, when mean temperatures were approximately 80°–85° F. by day and 55°–60° F. by night, and when plants were in a condition of vigorous growth (**4, 5**). Material for histological study was collected on July 21, 24, and 25. Flower buds, leaves, stems, underground stems, and roots were gathered. The underground parts were obtained to a depth of 14 inches, at which depth the main horizontal roots are found (**3**), since the watertable at Geneva is relatively high. Vigorous plants of sow thistle were treated on September 12, when mean temperatures were approximately 70°–80° F. by day and 55°–60° F. by night. The crown and 6 inches of vertical underground stem were collected on September 21 for histological study.

All material was fixed in formalin-acetic-alcohol, and the butyl alcohol-paraffin method of dehydration and imbedding was used. Sections were stained either with safranin and light green or with Flemming's triple stain modified by Margolena to include iodine.

[1] Journal Article no. 643 of the New York State Agricultural Experiment Station, Geneva, New York.

[2] This work was supported in part by a grant from the Dow Chemical Company, Midland, Michigan.

## Results

### Bindweed

Flower.—In gross appearance 1 day after treatment, the petals, stamens, and pistils of open flowers were brown, and anthers were shrunken and distorted. Flower buds and unopened flowers were arrested in development and failed to open.

The histological characteristics of the flower are similar to those of other members of the Convolvulaceae and need not be detailed here. Unopened flowers were used, since they provided unshed pollen as well as other floral parts still in development. The cells of the ovary wall were parenchymatous, and those in the developing ovules were meristematic. The cells of the nucellus were active and filled with starch grains. Starch was also found in moderate amounts in the sepals, petals, anthers, and pollen grains.

Histological changes were pronounced in the unopened flowers 7 days after treatment. The cellular structure of the sepals and petals seemed not affected, but the starch reserve, which was present in moderate amounts in those tissues, had disappeared by that time. Ninety-five per cent of the pollen grains had been affected, the cytoplasm having shrunken frequently from the walls—as if severe plasmolysis had occurred. A characteristic feature was the appearance of large vacuoles, as contrasted with smaller ones found in the pollen of untreated plants (fig. 1). Development of the endothecium of the anther was arrested. The cells appeared plasmolyzed, starch had disappeared, and cell contents were greatly reduced in density. In the ovule the nucellar tissue immediately surrounding the gametophyte differed markedly in untreated plants, the cytoplasm being much less dense. All meristematic activity in the nucellus had apparently ceased, and the cells had the appearance of mature parenchyma. Starch had disappeared from the entire ovule, except in the innermost layers of the nucellus. The cells of the ovary wall, although remaining more typically parenchymatous, underwent similar reduction in starch content.

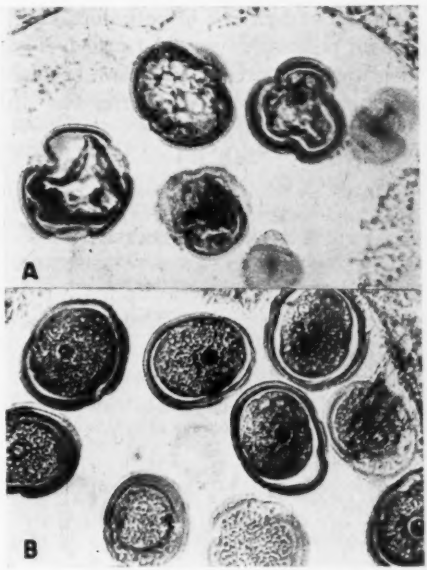


Fig. 1.—Effect of 2,4-dichlorophenoxyacetic acid on pollen grains of bindweed: *A*, pollen from treated plants, showing plasmolysis and large vacuoles. *B*, from untreated plants, showing turgid condition of pollen grains, and small vacuoles.

Leaf.—Within a few hours after treatment, the leaves in gross appearance were wilted and slightly folded upward along the midrib. After 24 hours the symptoms were more strongly evident, the plants having become dull green in color and lying flat to the ground. By the fifth day the basal leaves were yel-

low, and by the tenth day all leaves were withered and dead.

The leaves of untreated plants require no special mention, except that palisade cells occur on both the dorsal and ventral sides of the leaf. Minute grains of starch were observed in the chloroplasts.

Seven days after treatment the cells of the mesophyll and of both dorsal and ventral palisade layers had largely lost their dense contents and had become contracted, narrow and irregular in shape, and plasmolyzed. Chloroplasts were somewhat smaller and fewer in number than in untreated plants, but no reduction in starch content was observed. Many newly divided cells were found in the phloem region of the principal vascular bundles.

TABLE 1

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON SIZE AND NUMBER OF CELLS IN STEM OF BINDWEED, SEVEN DAYS AFTER TREATMENT

| Tissue | Average diameter of cells (μ) | | Approximate no. of rows of cells | |
|---|---|---|---|---|
| | Untreated | Treated | Untreated | Treated |
| Epidermal layer | 21 | 24 | 1 | 1–2 |
| Cortex | 52 | 53 | 11 | 11 |
| Phloem region | 15 | 15 | 6 | 10 |
| Cambial zone | 12 | 12 | 2–4 | 3–5 |
| Internal phloem | 15 | 12 | 5 | 8 |
| Pith | 42 | 66 | 9 | 10 |

STEM.—The principal gross responses of the stem to treatment were arrest in development, limpness, and loss of characteristic twining habit. Within 24 hours many climbing stems were hanging loosely, and others were lying flat to the ground.

The typical stem has a heavily cutinized epidermis. The cortex consists of a broad band of somewhat flattened cells containing scattered latex vessels which remain active throughout the life of the plant (7). At the inner part of the cortex is a conspicuous band of starch-filled cells, including the endodermis. Immediately within the endodermis is an intermittent ring of pericyclic fibers. A band of external secondary phloem is separated from the xylem by the cambial zone. The secondary xylem forms a complete or almost complete cylinder several layers in thickness, immediately adjacent to which are numerous islands of internal phloem. The two regions are separated by an intermittent band of internal cambium.

Response following treatment was varied. The epidermal and cortical cells were not greatly affected, although some cell division occurred in the subepidermal layer (table 1). The endodermal cells were activated to some extent, and both anticlinal and periclinal divisions were observed. The phloem and cambial regions showed activity within 24 hours of treatment, cell division occurring in both regions by that time. By 7 days after treatment the phloem region had become greatly activated and had increased in radial width 66% (table 1). The primary parenchyma had become crushed, and the pericyclic fibers were distorted and compressed and no longer stood out as a distinct ring. The cells of the cambial zone had also undergone stimulation by that time and had formed a multilayered band around the stem approximately twice as wide as in untreated stems. The mature xylem cells seemed not to have been affected. Internal phloem, however, underwent a change characteristic of treatment in both stem and rhizome. No longer serving as a storage region, the areas of phloem became actively dividing tissue. The meristematic

cells in these areas, being small, contrasted sharply with the larger parenchymatous cells of the pith, in which there was considerable enlargement of individual cells but no disorganization of the tissue (table 1).

RHIZOME.—The vertical rhizomes of bindweed develop from buds which are borne on the extensive horizontal root system. They are typically light-colored and somewhat woody and contain a great deal of storage material.

The gross response of the rhizome to treatment was in marked contrast to that of the above-ground parts. Five days after treatment, rhizomes were spongy, water-soaked, and enlarged to twice the diameter of similar portions of untreated plants. The outer layers showed longitudinal splitting and were easily sloughed off. The buds which are borne on the rhizomes failed to develop into shoots.

The structure of the typical untreated rhizome resembles that of the above-ground stem, although there is a much greater amount of secondary phloem and xylem, and pericyclic fibers are not found in the deeply buried portions of the rhizome (7). Sections cut 1–2 inches below the ground level contain only a few scattered fibers. The xylem is composed of a wide and almost continuous ring of large vessels, interspersed with small, thick-walled cells.

A considerable amount of starch is found in the rhizome, located mainly in the inner portion of the cortex. Parenchymatous cells of this region contain an abundance of large starch grains. Very little starch occurs in the outer portion of the cortex. The pith and ray cells contain a small amount of starch, although the individual grains are very small.

The response to treatment was more pronounced in the rhizome than in any other part of the plant (fig. 2). Individual cells of the periderm layer did not increase in size, but some cells in the subepidermal layer divided periclinally, resulting in a region three to four cells in thickness as compared with the 2- to 3-celled zone in untreated plants (table 2). The periderm, however, was ruptured and split in many places.

The cells of the outer portion of the cortex were greatly enlarged (table 2) and the walls frequently torn. The region increased in width radially from approximately 0.35 mm. to 0.60 mm. Pericyclic fibers, where present, were crushed and somewhat displaced. Except for the occasional fibers, there was no clear demarcation between the inner portions of the cortex and the phloem.

Starch disappeared gradually from the inner part of the cortex following treatment and had almost completely disappeared within 7 days (fig. 3). The grains which remained were clustered around the nuclei of the cells (fig. 3C). In some cases, however, starch disappeared after 1 day. This may have been due to the rapid action of the chemical, under optimum conditions.

Meristematic activity was markedly stimulated in the outer phloem and cambial zone. The phloem region increased radially from approximately 0.15 mm. to 0.30 mm. in width. A wide band of small active cells appeared after treatment, as shown in figure 2. In material collected 7 days after treatment, the cells of this entire activated zone contained dense cytoplasm and large nuclei, and they were easily distinguished from the adjacent cortical cells in which nuclei were small and often not seen in cross-section because of the large size of the cells.

Longitudinally, cells of the activated phloem region of treated plants were ar-

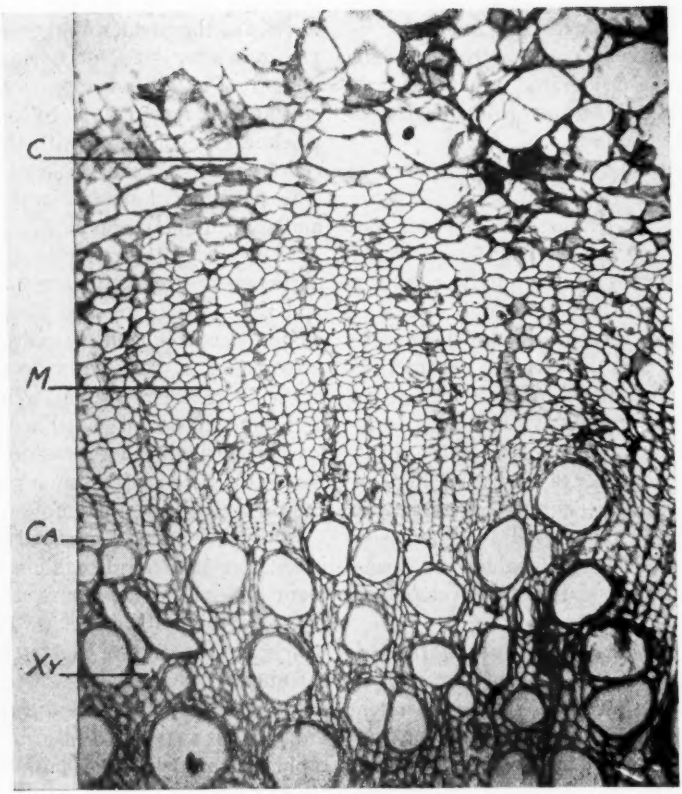


FIG. 2.—Typical response of rhizome of bindweed to treatment with 2,4-dichlorophenoxyacetic acid, showing activated meristematic region (*m*) external to several-layered cambial zone (*ca*) and xylem (*xy*) and enlarged cells of cortex (*c*).

TABLE 2

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON SIZE AND NUMBER OF CELLS AND ON RADIAL WIDTHS IN RHIZOME OF BINDWEED, SEVEN DAYS AFTER TREATMENT

| REGION | AVERAGE DIAMETER OF CELLS (μ) | | NO. OF ROWS OF CELLS | | RADIAL WIDTH OF REGION (mm.) | |
|---|---|---|---|---|---|---|
| | Untreated | Treated | Untreated | Treated | Untreated | Treated |
| Periderm | 43 | 43 | 3–5 | 3–5 | 0.1 | 0.15 |
| Cortex | 74 | 124 | 6–10 | 9–10 | 0.35 | 0.6 |
| Phloem region | 31 | 22 | 10–17 | 18–26 | 0.15 | 0.3 |
| Xylem vessels | 452 | 529 | 5–6 | 5–6 | 0.45 | 0.6 |
| Internal phloem | 18 | 16 | 10–12 | 8–14 | 0.1 | 0.2 |
| Pith | 47 | 68 | 4–5 | 4–6 | 0.25 | 0.3 |

ranged very regularly, and the typical long sieve tubes were few. This condition might indicate horizontal divisions in the phloem similar to those reported in *Iresine* after treatment with indoleacetic acid, in which long rows of cells were derived from a single parent phloem cell (**6**).

The internal phloem was also stimulated. As seen in cross-section, groups of phloem cells had formed clearly outlined, bulging, dome-shaped areas which developed inward, corresponding to similar areas in the stem. In the rhizome there were usually three main groups and one or two smaller ones, but the groups themselves were larger and more pronounced than those of the stem, almost completely filling the pith region. The pith cells immediately adjacent to these areas were compressed, so that a distinct layer of two or three rows of crushed cells often outlined the regions (fig. 4).

In general, the cells of the rhizome, especially those in the activated phloem region, were characteristically cytoplasmic and turgid, in sharp contrast to the cells of the flowers and leaves, which were highly plasmolyzed and vacuolate and showed no indication of becoming part of an actively dividing tissue.

ROOT.—The gross response of the root was similar to that of the rhizome but of less intensity. The roots increased somewhat in diameter and became spongy and water-soaked in appearance, accompanied by splitting and sloughing off.

The root has an outer corky tissue several layers in width. The root is an important storage organ, and the phelloderm and cortex comprise the main storage regions. The cells of the cortex especially are filled with many large starch grains. Within the cortex is a band of secondary phloem which contains many latex vessels (**7**). The xylem forms a solid cylinder in the center of the root

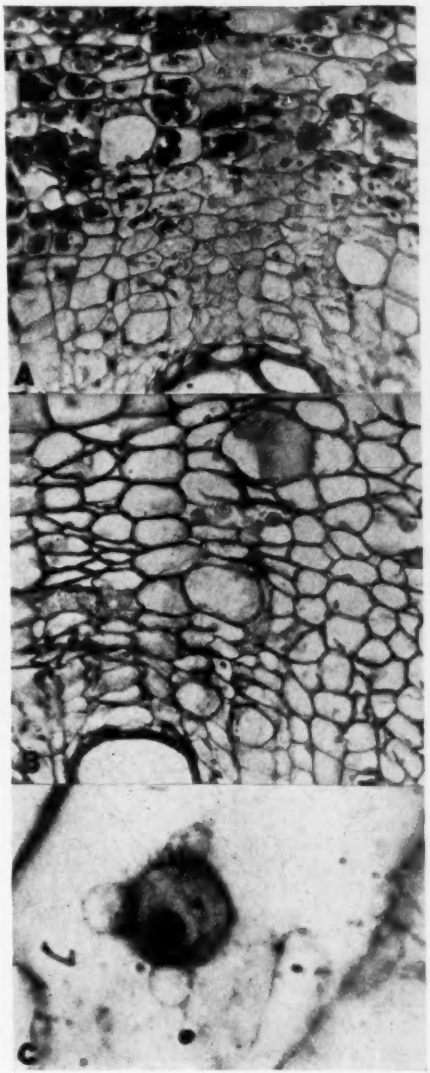


FIG. 3.—Effect of 2,4-dichlorophenoxyacetic acid on starch in rhizome of bindweed: *A*, portion of region in untreated rhizome showing abundance of starch grains in cortex and phloem regions. *B*, comparable area in treated region in which starch has entirely disappeared except for minute grains in one or two isolated cells. *C*, detail of cell showing the few remaining starch grains clustered around the nucleus.

and is composed of large vessels and many tracheids.

Histological changes in the main horizontal roots, 14 inches below the surface of the soil, were evident 7 days after treatment but were relatively small in comparison with changes in other portions of the plant. By that time the small parenchymatous cells near the large xylem vessels had divided, the cambial zone had become active, and the starch in nearby cells had partially disappeared. It is possible that activity in the roots would have continued for some time after the date of collection.

### Sow thistle

Sow thistle is a perennial weed reproducing by means of seeds and creeping roots. The horizontal roots, brittle and yellowish, grow in the upper 2–4 inches of the soil. A few thickened vertical roots may penetrate 5–10 feet. The rhizomes develop from buds on the horizontal

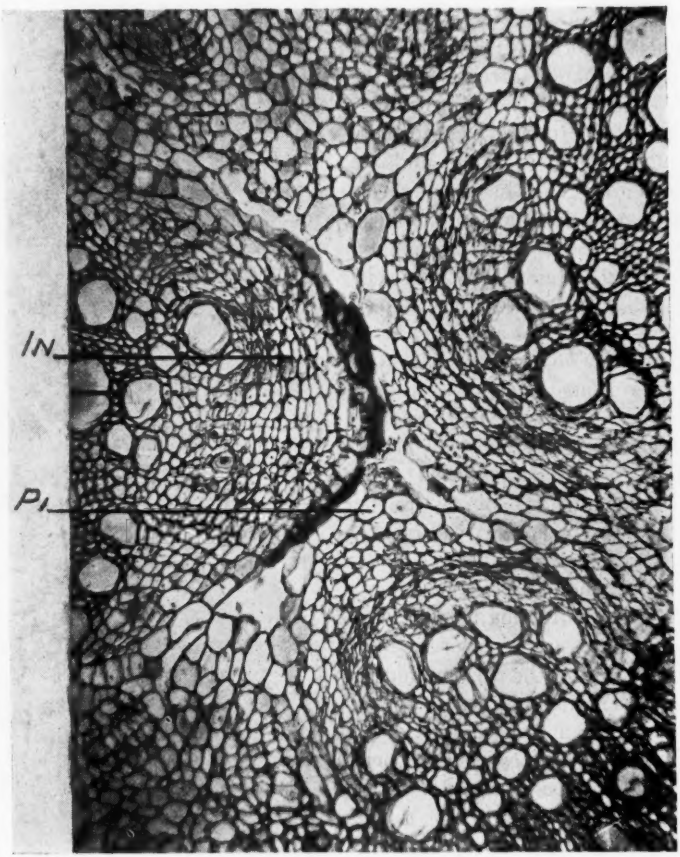


FIG. 4.—Detail of rhizome of bindweed treated with 2,4-dichlorophenoxyacetic acid, showing central region in which localized activity of internal phloem (*in*) has occurred, causing crushing in pith (*pi*).

roots and terminate in rosettes of light green leaves at the ground level.

The gross responses to treatment were rapid. Within 24 hours the leaves had become lighter green in color and slightly wilted. Two days after treatment the younger leaves were severely twisted and curled. After 10 days the outer leaves were progressively lighter green and decidedly wilted, while the central leaves showed extreme curvature. The bases of the inner leaves were enlarged and flattened, resembling the petioles of celery. The most conspicuous response was the large increase in diameter of the rhizome, ranging from 50% to 300%. All underground parts were soft and spongy and exhibited marked splitting of the outer regions. Entire plants were dead 2 weeks after treatment.

RHIZOME.—The typical rhizome is composed largely of parenchymatous cells (fig. 5*B*). At a point 1 inch below the crown of the plant, where sections were made, the periderm layer is three to four cells wide. The cortex is a narrow band of parenchyma, eight to twelve cells in width, containing a few scattered latex vessels. The endodermis, pericycle, and phloem region together constitute approximately one-fourth of the radius of the rhizome. The endodermis is intermittent and indefinite, so that the parenchyma of the inner region appears to be continuous with that of the cortex. Differentiated phloem occurs in narrow radial strands separated from one another by relatively broad bands of parenchyma. Lactiferous ducts and groups of medullary phloem which are in close con-

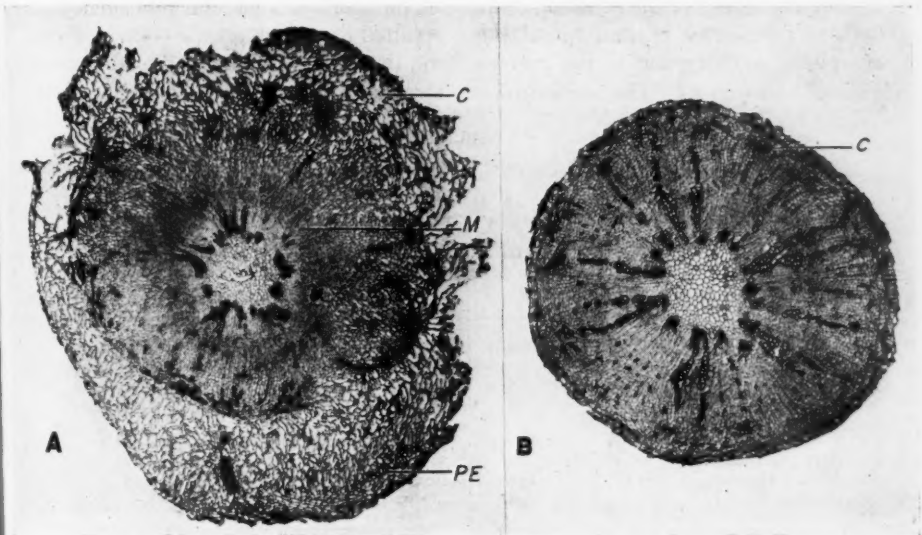


FIG. 5.—Typical response of rhizome of sow thistle to treatment with 2,4-dichlorophenoxyacetic acid: *A*, rhizome showing increase of cortex (*c*) to several times usual size, due to cell enlargement; formation of secondary periderm (*pe*) in cortex; and loss of identity of strands of secondary phloem in activated region (*m*) within intermittent ring of compressed latex vessels and endodermis. *B*, rhizome from untreated plant, showing relatively small cortex (*c*) and distinct radial strands of xylem and phloem.

junction are scattered throughout the region. The xylem constitutes nearly one-third of the radial diameter and, like the phloem, occurs in radial strands between which are broad bands of parenchyma. The pith is relatively small, being approximately one-fifth the radius of the rhizome.

There was much disorganization in most of the tissues of the rhizome, owing both to an increase in, and stimulation of, cellular activity and to the enlargement of cells (fig. 5*A*). The periderm was less degree than in the cortex. The regions increased in radial width from 0.5 mm. to 0.8–1.0 mm. In the outer part of the phloem region many cells had doubled in diameter, and the latex cells tended to become indefinite in form. Toward the inner part of the region there was much cell division but no pronounced cell enlargement. The greatly increased activity of the cambial zone and phloem region resulted in a disorganized mass of dividing tissue, and the identity of the radial strands of phloem was completely lost.

TABLE 3

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON SIZE AND NUMBER OF CELLS AND ON RADIAL WIDTHS IN RHIZOME OF SOW THISTLE, NINE DAYS AFTER TREATMENT

| Region | Average diameter of cells ($\mu$) | | No. of rows of cells | | Radial width of region (mm.) | |
|---|---|---|---|---|---|---|
| | Untreated | Treated | Untreated | Treated | Untreated | Treated |
| Periderm | 62 | 68 | 3–6 | 2–4 | 0.1 | 0.1 |
| Cortex | 58 | 128 | 8–12 | 10–12 | 0.2 | 0.9–1.4 |
| Pericycle and phloem region | 21 | 17–54 | 18–22 | 26 | 0.5 | 0.8–1.0 |
| Cambial zone | 20 | 16 | 3–4 | 3–5 | 0.15–0.2 | 0.25 |
| Xylem vessels | 169 | 135 | 16–20 | 19–20 | 0.5 | 0.4 |
| Pith | 55 | 58 | 8–10 | 8–10 | 0.5 | 0.6 |

ruptured in many places, and cells of the cortex were greatly enlarged, especially in a radial direction, to two to ten times the usual width (table 3). Individual cells occasionally measured approximately 200 × 300 $\mu$, as compared with approximately 20 × 50 $\mu$ in an untreated plant. The cortical region as a whole increased in width from approximately 0.2 mm. to 0.9–1.4 mm. The resulting increased size of the cortex became the most conspicuous feature of treated plants. Secondary periderm layers were occasionally formed in the disorganized cortex.

Cells of the pericyclic and phloem regions were distorted, although to a much

Many lateral roots observed in treated material may have been initiated by the treatment. The pith was relatively less affected than other portions of the rhizome, although there was occasional rupturing of the tissue.

STARCH.—A striking and characteristic response to treatment was the disappearance of starch grains in portions of the flower, stem, rhizome, and root. With the exception of the flower, starch disappearance was correlated with new growth of cells. Starch grains were fissured, angular, and smaller in size in storage cells nearest the regions of stimulated activity. In the flower, starch disappeared from the petals, anthers, and ovary in the ab-

sence of cell division, although its disappearance may have been correlated with the development of tissues along other lines. The leaves did not show pronounced acceleration of starch digestion after treatment, although the chlorotic appearance of the leaves indicated that the function of the chloroplasts had been impaired.

Since treatment in most instances promoted the disappearance of starch from the cells of living plants, tests were made of the effect of 2,4-dichlorophenoxyacetic acid on starch *in vitro*. For this purpose, 1% starch solution was prepared with Argo cornstarch. Ten cubic centimeters of the solution was placed in each of five test tubes: one remained as a control, while the acid was added to each of the other four in concentrations of 1 mg./l, 10 mg./l, 100 mg./l, and 1000 mg./l. One-tenth cubic centimeter of iodine solution (0.3% iodine and 1% potassium iodide) was added to each test tube. This was followed by 5 cc. of a 1% solution of taka-diastase. A temperature of 25° C. was maintained during the experiment. The color change in each test tube was recorded with a Lovibond colorimeter at 1-minute intervals up to 10 minutes.

The results given in table 4 fail to show any acceleration of starch hydrolysis with the acid beyond the normal rate of hydrolysis of the control. On the contrary, at 100 mg./l, and especially at 1000 mg./l, there was marked inhibition of hydrolysis. This same reaction might not necessarily occur in the plant, however, as there are many other factors involved in plant processes.

TABLE 4

EFFECT OF VARIOUS CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID ON HYDROLYSIS OF STARCH WITH TAKA-DIASTASE, AS MEASURED BY LOVIBOND COLORIMETER. LOSS OF COLOR* INDICATES HYDROLYSIS OF STARCH

| Concentration | Color | No. of minutes after introduction of taka-diastase | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Control | Red | 3.2 | 3.1 | 2.9 | 2.4 | 2.2 | 1.8 | 1.7 | 1.5 | 1.3 | 1.3 | 1.2 |
| | Blue | 5.0 | 4.5 | 4.2 | 3.4 | 3.2 | 2.7 | 2.5 | 2.4 | 2.2 | 2.0 | 1.9 |
| 1 mg./l | Red | 3.0 | 2.8 | 2.2 | 2.0 | 1.7 | 1.4 | 1.4 | 1.4 | 1.2 | 1.1 | 1.0 |
| | Blue | 5.5 | 4.8 | 3.8 | 3.3 | 3.1 | 3.1 | 3.1 | 3.1 | 2.9 | 2.3 | 2.1 |
| 10 mg./l | Red | 2.6 | 2.4 | 2.3 | 2.1 | 1.9 | 1.8 | 1.4 | 1.3 | 1.3 | 1.2 | 1.2 |
| | Blue | 4.6 | 4.3 | 3.8 | 3.5 | 3.2 | 3.0 | 2.9 | 2.6 | 2.6 | 2.2 | 2.2 |
| 100 mg./l | Red | 3.2 | 3.1 | 2.4 | 2.4 | 2.2 | 2.1 | 2.1 | 1.5 | 1.4 | 1.4 | 1.4 |
| | Blue | 5.4 | 5.0 | 4.8 | 4.2 | 3.7 | 3.3 | 3.2 | 3.1 | 3.0 | 2.9 | 2.7 |
| 1000 mg./l | Red | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 | 3.1 | 3.1 | 3.1 | 3.1 | 3.1 |
| | Blue | 5.5 | 5.3 | 5.2 | 5.2 | 5.2 | 5.2 | 5.1 | 5.1 | 5.0 | 4.9 | 4.7 |

* Numbers indicate intensity of color.

## Discussion

Many of the responses induced by the action of 2,4-dichlorophenoxyacetic acid on bindweed and sow thistle resemble those reported by KRAUS *et al.* with indoleacetic acid on bean (**8**). This acid, however, produces a much greater effect in more dilute concentrations, and it also produces a marked response at some dis-

tance from the point of application, traveling a considerable distance in the underground system. The distance that the acid travels is important, since regeneration of the plant may occur from underground buds if these are not injured.

The primary object of the present study was to determine the histological changes which accompanied death of bindweed and sow thistle following treatment with 2,4-dichlorophenoxyacetic acid and to attempt to correlate these changes with the death of the plants.

In the case of bindweed, the effect of the acid on immature pollen grains and on developing tissues of the ovule becomes important, in view of the propagation of the plant by seeds. When 95% of the pollen becomes functionless, the probability of fertilization is greatly reduced, the arrested floral development precluding the development of seeds.

The chlorotic appearance of the sprayed leaves suggests that the manufacture of chlorophyll in the chloroplasts is impaired, and the division of phloem cells of the larger vascular bundles of the leaf suggests a possible disturbance in the normal movement of food from the leaf.

Increased cell division, which was an early symptom of response, especially in the rhizome, occurred first and was greatest near the large xylem vessels. This fact might indicate that the acid or some substance stimulated by it was transported through the plant in these vessels. As in the leaf, the phloem of stem and rhizome—being in close proximity to the xylem elements—underwent marked stimulation, which might easily result in a major disturbance in the transfer of food. Such a condition would be of vital importance to the life of the underground regions, where no food is manufactured.

An increase in meristematic activity also indicates an increase in the rate of respiration and the further depletion of reserves, which has been shown to be an important factor in weed control (1, 10).

The enlargement and proliferation of cells in the rhizome were accompanied by disorganization and rupturing of cells and tissues. The tearing of the epidermis and periderm in many places provided an easy means of entry for fungi and bacteria. Since starch was found to disappear from many regions, there is the possibility of an increase in available sugars, which would be favorable to the growth of organisms and to the final decay and disintegration of the plants.

In a general sense, the changes in the rhizome of sow thistle were not unlike those in the rhizome of bindweed. The greatly increased cell division in the phloem and cambial regions and the marked increase in size of cells of the cortex resulted in disorganization and rupturing of cells and tissues, which not only markedly alter the appearance but also upset the normal functions of the plant, and so offer opportunity for microörganisms to bring about disintegration and decay.

## Summary

1. Bindweed and sow thistle were treated with aqueous sprays of 2,4-dichlorophenoxyacetic acid at 1000 p.p.m. in 0.5% Carbowax 1500, applied during midsummer while the plants were growing vigorously. The responses of the plants were studied and the tissues examined to correlate histological changes with the death of the plants.

2. In bindweed, pollen grains were plasmolyzed and disorganized, flowers were arrested in development, chlorophyll formation was checked, and cell division was activated in the large vascular bundles of the leaves. The majority of the cells in the leaves were plasmolyzed.

3. Cell division was greatly increased in all cambial zones and phloem regions of the stem and rhizome of bindweed. Enlargement and rupture of cortical cells of the rhizome were conspicuous features.

4. The root of bindweed responded more slowly to treatment than did other parts of the plant, so that changes which had occurred 7 days after treatment were of less intensity than in the rhizome but similar in nature.

5. Starch disappeared from almost all parts of the flower of bindweed, but very little hydrolysis of starch occurred in the chloroplasts of the leaves. Disappearance of starch from the endodermis of the stem and the inner cortex of the rhizome and root was correlated with active cell division in the phloem region of those portions of the plant.

6. In the rhizome of sow thistle, cells of the cortex were greatly enlarged and frequently torn. The periderm was ruptured, and disorganized large-scale cell division occurred in the cambial zone and phloem regions.

7. Starch hydrolysis was inhibited *in vitro* by the action of the acid.

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# DEVELOPMENT OF SPORE-FORMS AND THE NUCLEAR CYCLE IN THE AUTOECIOUS OPSIS RUST, CYSTOPSORA OLEAE

M. J. THIRUMALACHAR

## Introduction

The principal diagnostic characters of the genus *Cystopsora*, founded by BUTLER (9) to accommodate the rust on *Olea dioica* Roxb., are the occurrence of basal cells or cysts in the telia and the germination of the teliospores without a rest period. The telia are superstomal, and the teliospores—which germinate *in situ*—form a semi-internal, 2-celled promycelium, each promycelial cell bearing a sessile sporidium. BUTLER found a few aecia associated with the telia, and he tentatively interpreted them as the aecial stage of the rust. AJREKAR (1), who carried out infection tests, confirmed the interpretation of BUTLER and showed that the rust is an autoecious opsis form. *Cystopsora oleae* Butl. has been reported from Ceylon by PETCH (21) on *Olea polygama* Wight, and SYDOW (24) has recently established a second species, *C. notalaeae*, on *Notalaea longifolia* Vent. from Australia.

Abundant living material of this rust became available in 1940 at the Coffee Experiment Station, Balehonnur, Mysore State, and a careful examination of it showed that the promycelia, instead of being 2-celled, were in fact 4-celled. This provided the stimulus for undertaking a detailed study in order to follow the various nuclear phases of the species. The investigation has revealed that not only are the promycelia 4-celled, but that the sequence of life cycles is very unusual. In fact, the type of development is so remarkable, and so much at variance with accepted concepts regarding the function and the sequence of the various spore-forms, that it has been considered worth while to place on record all the various carefully made observations in the course of this investigation. Opportunity has also been taken to emend the diagnosis of the genus and the species, to conform to the new facts now brought to light.

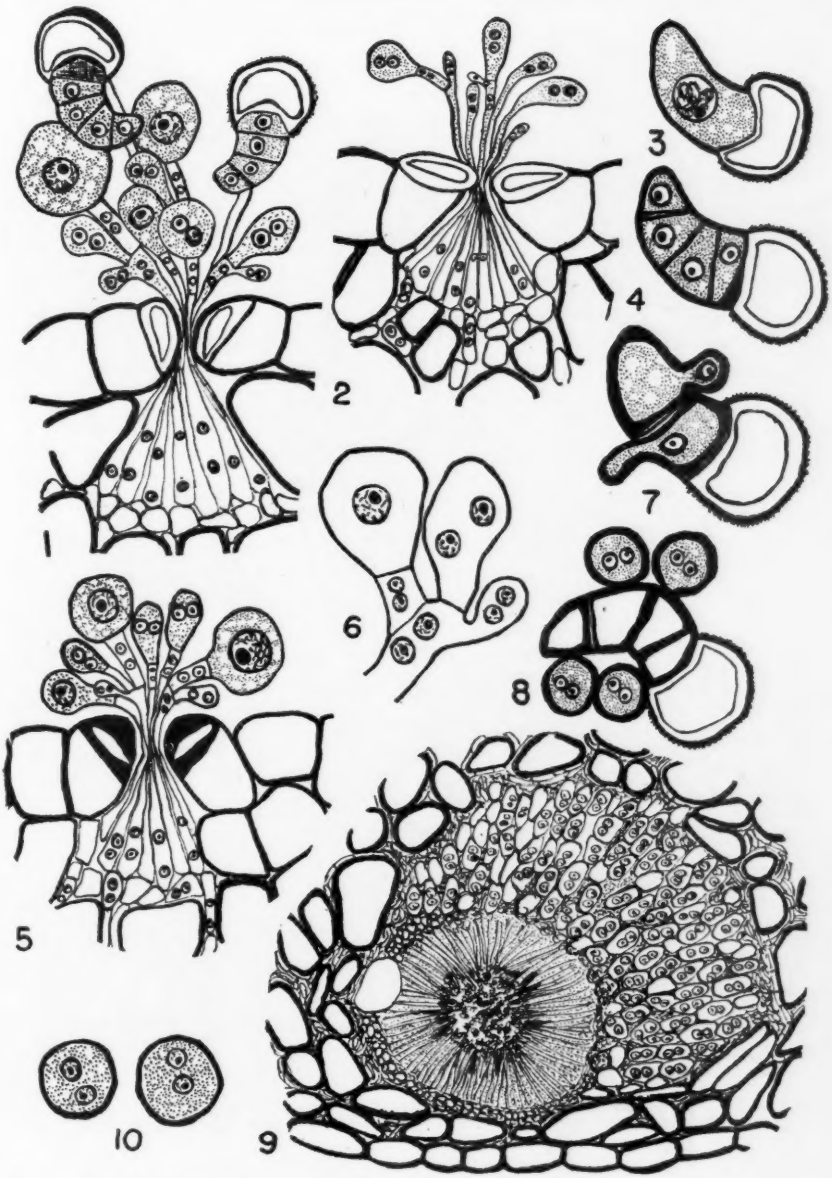
METHODS.—Material for the study was fixed in three fluids—Bouin's as modified by Allen, Craf, and 2BD. Heidenhain's iron-alum haematoxylin with eosin in clove oil was generally employed for staining the spores; but for critical studies, especially when the nuclear phase of the mycelium and of the spores had to be confirmed, Feulgen's stain was used. Delafield's haematoxylin and safranin combinations were employed with good effect for studying the pathological anatomy.

## Observations

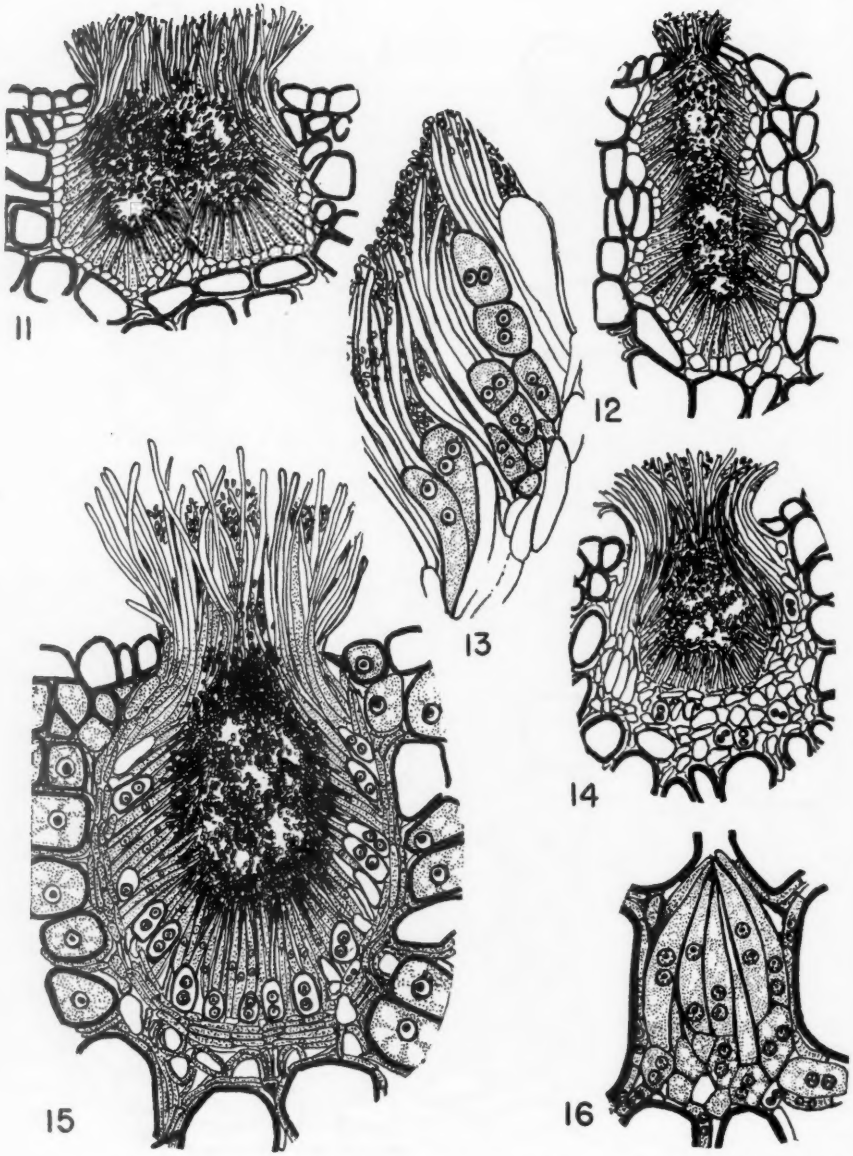
### SPORE-FORMS

The rust occurs on the host throughout the year in one or the other spore-form. Pycnia and aecia are distributed on the leaves and tender shoots, which become much hypertrophied. When the axillary buds are infected, especially their meristem, large "witches' brooms" studded with pustules are formed. On their being shaken, clouds of aeciospores are shed. Later the witches' brooms dry up but the mycelium perennates in the basal region. When fresh growth takes place, the newer outgrowths are completely covered with the sori.

PYCNIA.—These are subepidermal and flask-shaped, with well-developed ostio-




FIGS. 1–10.—Figs. 1, 5, section through telium. Fig. 2, through young telium. Figs. 3, 4, germination of teliospores. Fig. 6, development of teliospores in clusters. Fig. 7, 2-celled promycelium. Fig. 8, germinating teliospore with sessile sporidia. Fig. 9, differentiation of pycnium and aecium from same plectenchyma. Fig. 10, binucleate sporidia.




FIGS. 11–16.—Fig. 11, coalescing pycnia. Fig. 12, coalescence of superposed pycnia. Fig. 13, abstriction of aeciospores from basal cells beneath pycnium. Fig. 14, development of plectenchyma within pycnium. Fig. 15, subepidermal pycnia. Fig. 16, binucleate pycnial hyphae and basal cells.

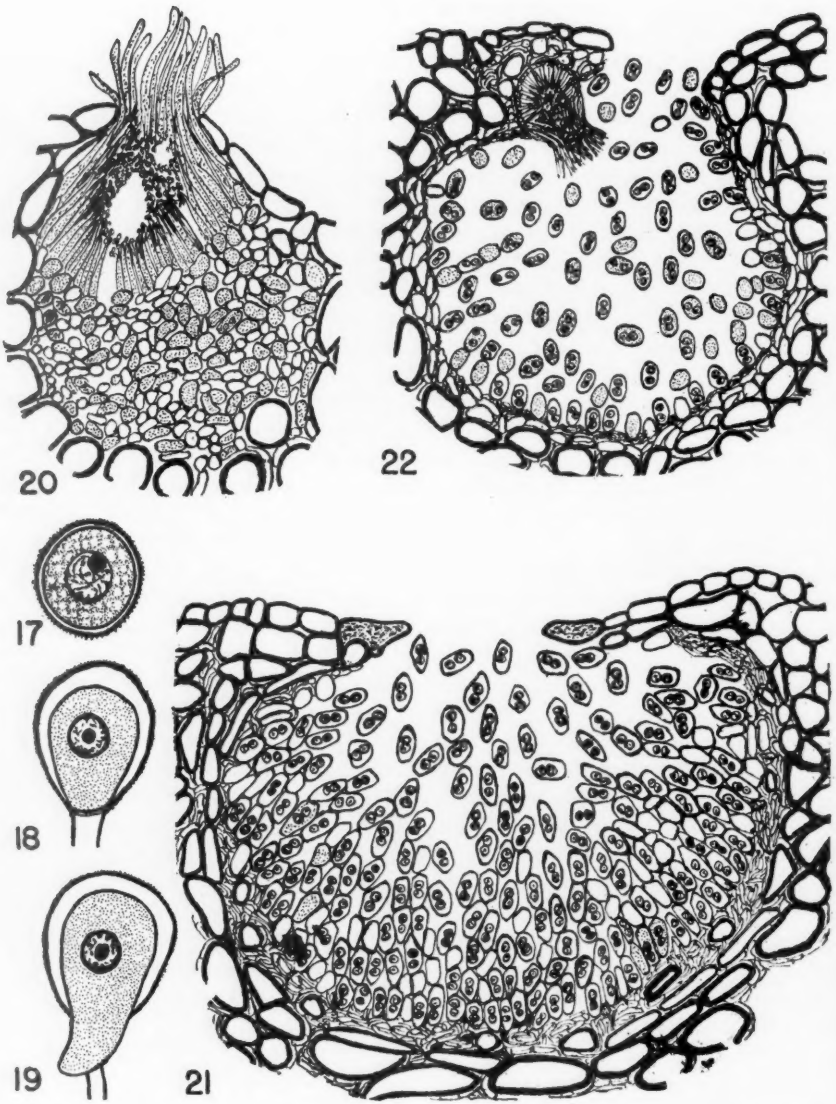
lar filaments (fig. 15). They are amphigenous and can be differentiated from the aecia by their orange-yellow color. They occur in abundance, both on the leaves and on the hypertrophied portions of the witches' brooms but are soon displaced by the aecia. When two pycnia occur close together, they coalesce (fig. 11), and in some cases they may even become superposed in such a manner that the ostiole of the lower pycnium fuses with the basal region of the one above, resulting in the formation of a continuous chamber (fig. 12). In such cases the spermatia from both pycnia emerge through a common ostiole.

AECIA.—These are amphigenous, erumpent, and cupulate. They are yellow, in contrast to the orange-yellow color of the pycnia. No peridium surrounds them. The aeciospore chains, like the pycniosporophores, converge toward the center, however, rather than form vertical rows (fig. 21). This type of formation of aeciospore chains has evidently eliminated the necessity of a peridial wall. When in close proximity, the aecia may also coalesce, and the fusion of aecial cups that are superposed has been noted (fig. 27). Aeciospores are yellow, binucleate, ovate-elliptic to fusiform, deeply angular, with distinct germ pores. The germ pores become rather prominent at the time of germination. Their exospore is sculptured with reticulations which become distinct when the spores are mounted in plain agar. They can be germinated without difficulty and stained by the method suggested by the writer (**26**).

TELIA.—The telia are hypophyllous, pulverulent, and sparsely distributed on the undersurface of the leaves. When close together they also coalesce, forming concentric rings. They emerge through the stomatal aperture (figs. 1, 5), but the infection patches are not hypertrophied. The hyphae passing out of the stoma are distinctly separate (fig. 2), and this feature is observable in the early stages. The tip of the emerging hypha becomes rounded off, and a binucleate spore initial is cut off from which the stalk cell and the teliospore initials are fashioned. By repeated division the basal cell forms successive spore initials, which in turn form more stalk cells and teliospores (fig. 6). The teliospores are therefore formed in basipetal succession by the activity of a single generative cell. When a branch is separated by manipulation under the microscope, clusters of spores at different stages of maturity and attached to the basal generative cell can be clearly observed. In a mature telium the basal cell becomes expanded, forming a discoid apex. It is manifest that BUTLER mistook the fascicular basal cells for cysts and, on this basis, discussed the affinities of the genus *Cystopsora* with *Ravenelia*, *Uromycladium*, and others where the development of cysts is a well-established character. In fact, the structure referred to by BUTLER is the discoid basal generative cell. In this respect the genus shows affinity toward *Chaconia* or *Scopella*—genera in which the teliospores develop in clusters from laterally free generative cells. The young teliospores are binucleate, but later a syncaryon is formed (fig. 17). The mature teliospores are minutely verrucose and very much thickened, but only over the distal two-thirds of their circumference. The proximal third is smooth and thin, and the stalk is inserted into the thin portion—usually laterally.

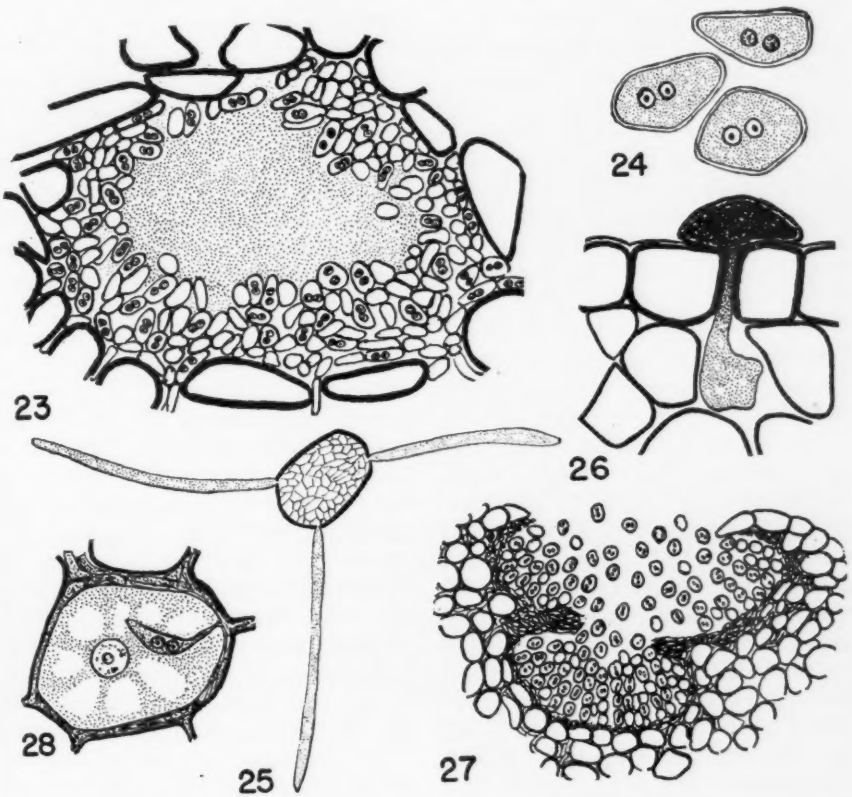
## GERMINATION

The teliospores germinate within the telium as soon as they are mature and while still attached. There is no germ pore, but the proximal thin-walled por-




FIGS. 17–22.—Fig. 17, mature teliospores with syncaryon nucleus. Figs. 18, 19, germinating teliospores showing position of pedicel. Fig. 20, plectenchyma replacing pycnosporophores within pycnium. Fig. 21, aecium. Fig. 22, pycnium in margin of aecial cup.

tion grows out into a blunt process. The promycelium grows by the side of the stalk, usually bent at an angle, and its contents develop a slightly orange tint. The first wall is cut off within the teliospore itself, and in most of the cases—as found that, while the 4-celled promycelium is the rule in *Cystopsora* (fig. 8), a 2-celled promycelium also occurs (fig. 7). Examination of the type material of *Cystopsora oleae* deposited in the Herbarium Cryptogamie Indiae Orientalis




FIGS. 23–28.—Fig. 23, aecial plectenchyma. Fig. 24, aeciospores. Fig. 25, aeciospore germination. Fig. 26, penetration of cuticle by germ tube of aeciospores. Fig. 27, coalescence of superposed aecia. Fig. 28, haustorium within host cell.

the promycelium elongates—it carries along with it the stalk also (fig. 19). In later stages the stalk can be seen attached to the second or the third promycelial cell. BUTLER stated that the 2-celled promycelium is the most distinguishing feature of this rust; but it has now been revealed that in most of the teliospores in the material collected at Khandala by CHIBBER, and at Belgaum by AJREKAR, the teliospores had germinated by forming a 2-celled promycelium, but a few 4-celled promycelia have now been discovered by careful examination. CHIB-

BER collected in July, 1909; but a second collection made in the same locality in December, 1941, by Rev. H. SANTAPAU had equal numbers of 2-celled and 4-celled promycelia. In the collections made by the writer in Mysore, 4-celled ones have always predominated. The 2-celled promycelial condition is apparently due to the failure of cross-wall formation, but the exact factors which have led to such a failure are not exactly understood. In the closely allied monotypic genus *Zaghouania*, in which the promycelia are as a rule 4-celled, DIETEL (11) reports that 2-celled promycelia also occur when conditions are not very favorable.

## CYTOLOGY

Each young teliospore has one pair of conjugate nuclei, but the two nuclei fuse and form a syncaryon in mature spores. As the promycelial initial begins to develop, the syncaryon moves into the promycelium (fig. 3) and undergoes two successive divisions accompanied by wall formation, ultimately resulting in a 4-celled promycelium, each cell of which has a single nucleus (fig. 4). The sporidia are somewhat thick-walled, with orange-colored contents. They do not germinate *in situ*. While each of the promycelial cells is uninucleate, all the sporidia observed had two nuclei (fig. 10). Hundreds of preparations have been examined, and while the exact dividing process of the sporidial nucleus has not been observed, there is no doubt about the binucleate condition of the sporidia. As far as the observations of the writer go, the mycelium bearing the pycnia, aecia, and telia has been found to be binucleate. The uninucleate condition has been observed only in the mature teliospore after the syncaryon has formed in the 4-celled promycelium and in the pycniospores. There appears to be, therefore, sufficient justification for considering the binucleate condition in the sporidium and subsequently in the mycelium as the diplophase.

Precocious division of the nucleus in the sporidium has been observed by others. In *Puccinia malvacearum* Bert., ASHWORTH (3), ALLEN (2), and SAVILE (23) have shown that the sporidium nucleus divides before germination of the spore; and the same appears to be true, according to SAVILE, in *Uromyces lespedezae-procumbentis* (Schw.) Curt. and *Melampsora bigelowii* Thuemen. SAVILE even found a quadrinucleate sporidium in the former species. The binucleate sporidia of *Puccinia malvacearum* give rise on germination to a uninucleate mycelium, but binucleate cells again appear during the development of the sorus plexus. The sporidia of *Endophyllum sempervivi* (Alb. & Schw.) de Bary are likewise binucleate, and ASHWORTH (4) suggests that, as the nuclei have to pass (while penetrating the host) through a small aperture, it is easier for two smaller nuclei rather than one large nucleus to do so; if in such a process one gets damaged, the other carries on the development. But in these rusts the binucleate hyphae later become uninucleate. This, however, is not the case in *Cystopsora oleae*. Division of the nucleus takes place even before the sporidium has started to germinate, and the persistence of this binucleate condition becomes manifest in the pycnial initials and the hyphae composing them (fig. 16).

Experiments on infection were conducted in order to follow the subsequent history of the nuclei. Young leaves of *Olea dioica* were sprayed with water and dusted with sporidia and the plants placed in a moist chamber. Bits of inoculated leaves were fixed at intervals of

8 hours for 3 days and then sectioned and stained in the usual manner. It was noted that the germ tubes from the sporidia penetrate the host cells through the cuticle and ramify in the intercellular spaces. In early stages they remain multinucleate without the formation of septa, and as many as thirty nuclei have been counted in a hypha at some distance from the tip. As development proceeds, the nuclei become grouped in pairs or fours and are later separated by septa. This type of development has been observed by HIRMER (16) in *Psalliota campestris* (L.) Fries. The pycnial mycelium, while showing binucleate hyphae for the most part, consists of multinucleate cells also. The pycnial initials are subepidermal and concentrated in the intercellular spaces.

A situation more or less akin to this occurs in *Puccinia arenariae* (Schum.) Winter, which was investigated by LINDFORS (20), an account of which is given by JACKSON (17). In this rust the two nuclei fuse in the usual way and the fusion nucleus passes into the promycelium, where it divides, the division being followed by a cross-wall formation between the daughter nuclei. But when the nuclei divide again no cross walls are formed, so that the promycelium is 2-celled, each cell having two nuclei. Later the two nuclei pass into the sporidium, which is throughout binucleate, so that the diplophase is initiated by the failure to form cross walls in the promycelium.

But in both *Puccinia malvacearum* and *P. arenariae*—the forms in which the diplophase is initiated in the sporidium itself—there is no pycnium formation. According to JACKSON (17) the pycnia always develop on the haploid thallus, the pycniospores being uninucleate. They are always present in the eu-forms, whether heteroecious or autoecious; however, they are likely to be absent in opsis forms that appear to be transitional in character. In a great majority of microforms they are stated to be absent, especially in those with a predominantly binucleate mycelium.

The presence of well-developed pycnia on what in fact is a diploid mycelium in *Cystopsora oleae* is therefore a phenomenon at variance with accepted concepts. JACKSON (17) is of the opinion that the presence of pycnia indicates that the rust is heterothallic and that the absence or under-development of pycnia would indicate that the rust is homothallic. BROWN (5), who showed that *Puccinia coronata elaeagni* Fraser & Ledingham produces abortive pycnia occasionally and that *P. grindeliae* Peck produces them only on one of its hosts, holds the same view. Even though monosporidial infection experiments have not been carried out on *Cystopsora oleae*, the initiation of the diplophase in the sporidium indicates that this rust is also homothallic. The pycnium has, since the classical work of CRAIGIE (10), been interpreted as a structure having to do with sexuality in rusts. In homothallic microcyclic forms, JACKSON (18) states that the pycniospores may no longer be functional. That may be the case in *C. oleae*, but the formation of these structures on a diploid mycelium suggests that the sexual function is not a necessary attribute of the pycnium.

In a previous paper, the writer (25) has reported the occurrence of aeciospores and teliospores within the pycnial cups of *Uromyces hobsoni* Vize. According to JACKSON (17), the potentiality is genetically present for any given species for the immediate production of any spore-form which occurs in its life history as soon as the change from the haploid to the diploid phase has taken place,

since such forms are sooner or later produced by the mycelia descended from the fusion cells. Occurrence of aeciospores and teliospores in the pycnia of *U. hobsoni* is thus clear, for the mycelium had entered the diplophase in the sporidium itself. In *Cystopsora oleae* the formation of aeciospores in the pycnial cups has also been noted (figs. 13, 14, 15). In young pycnia each basal cell possesses two large nuclei of the expanded type, as classified by SAVILE (23), and from such cells aeciospores are abstricted in chains of two or three into the cavity of the pycnium. Later, in many cases the basal cells (other than those that have enlarged) multiply actively, grow into the cavity of the pycnium, and gradually displace the pycniosporophores (figs. 14, 20). Various stages in the development of this plectenchyma have been observed. In some cases only the ostiole and a few pycniosporophores remained in the original pycnium, while in others the entire contents became displaced.

While observing the development of the aecia, the development of a small pycnium from the hyphae lining the margin of the aecium has been noted (fig. 22). In another instance both the pycnium and the aecium were seen to differentiate from the same plectenchyma mass, part of the initial having formed the pycnium and part the aecium (fig. 9). Such a mode of development is to be expected, as the mycelium resulting from the sporidia is already diploid.

In the rusts that have so far been investigated—such as *Puccinia arenariae*, *P. anemones-virginianae*, and *P. heucheriae*—JACKSON (18) states that the diplophase is initiated in the sporidium itself but the promycelium is 2-celled owing to the failure to form cross walls. Such a failure occurs also in *Cystopsora oleae*, for the collections of rusts made at Khandala and Belgaum and studied by BUTLER (9) had a majority of promycelia with two cells. The rust is apparently in an unstable condition; and instead of a 4-celled promycelium, with the consequent necessity for a third division of the nucleus in the sporidium, it is probably tending to develop 2-celled promycelia, so that only two divisions of the syncaryon suffice. Rather than a case of arrested development, the 2-celled promycelium may be an advance suited to the peculiar nuclear history of this rust.

The two nuclei in the sporidium which are evidently conjugate nuclei are held by JACKSON (18) to be chromosomally alike. But they should be considered as having sexually differing potentialities, and BULLER (7) suggests that such nuclei have a mixed sex tendency, basing this view on the work of HANNA (13, 14), HARDER (15), and SASS (22).

## EXPERIMENTS ON INFECTION

Experiments carried out to confirm the autoecious nature of the rust, which had already been established by AJREKAR (1), would not call for particular mention but for the fact that further complexities in the life cycle have been encountered.

For these experiments, test plants were grown in pots under rust-free conditions. Young leaves were sprayed with water and then inoculated with fresh sporidia and the plants placed in moist chambers. Infection spots became visible on the fourth day as dark green specks on the pinkish leaves. With the gradual enlarging of the spots the infected portions became hypertrophied, resulting in a malformed condition of the leaves. Pycnia were seen on the tenth day as orange-colored specks, and young aecia became visible on the infection spot 13

days after infection, masses of aeciospores beginning to erupt after rupture of the epidermis.

When aeciospores became available, young leaves of the host plant were sprayed with water, dusted with aeciospores, and placed in a moist chamber for 48 hours. As with the sporidial infection, the spot became visible on the fourth day and spread into a hypertrophied patch. The first spore-form to develop on this malformed structure was —strangely enough—a pycnium, which later became displaced by the aecium. The aeciospores from aecia produced by secondary infection were collected and again used for infecting young leaves of *Olea dioica*. The same symptoms became manifest, and pycnia were again produced as with the first spore-form, these later becoming displaced by the aecia. Such a series of tests with aeciospores of the previous generation was continued up to six generations, with identical results.

It is manifest that the aeciospores of *Cystopsora oleae* have assumed the role of sporidia, in that they develop pycnia on infecting young leaves of *Olea dioica*. The aeciospores never germinate by the formation of sporidia as in some of the microcyclic rusts that have been investigated. In *Endophyllum sempervivi*, for instance, the aeciospores always germinate by the formation of a promycelium with basidiospores and are usually termed telioid aeciospores. In *Kunkelia nitens* (Schw.) Arth., DODGE (12) pointed out that normal aeciospores and telioid aeciospores are associated within the same sorus. In *Gymnoconia interstitialis* Lagerh., on the other hand, where normal two-celled teliospores also occur, KUNKEL (19) pointed out that the aeciospores in certain seasons germinate by the formation of a promycelium with basidiospores.

Recently, BROWN (6) has shown that when the urediospores of *Puccinia minussensis* Thuemen are sown on seedlings of *Lactuca pulchella*, secondary uredia develop in abundance but the infection later becomes gradually systemic and spreads toward the base, into the rhizome. When these rhizomes are removed and replanted in pots, the emerging shoots are studded with pycnia. BROWN has shown, however, that a de-diploidization of the diploid mycelium takes place, resulting in the formation of haploid pycnia and haploid protoaecia. The same phenomenon has been demonstrated in *Puccinia suaveolens* (Pers.) Rostrup by BULLER and BROWN (8). BULLER therefore concludes that in rust fungi with systemic mycelia that perennate in the subterranean organs of herbaceous hosts, de-diploidization of the diploid mycelium takes place annually.

Evidence of such de-diploidization has not been seen in *Cystopsora oleae*, for even the pycnial initials are binucleate, and a haploid mycelium has not—as already stated—been observed in the numerous preparations examined.

The experiments on infection have been carried out at different times of the year, using aeciospores, and it is beyond doubt that young leaves, irrespective of the season, invariably develop pycnia as the first spore-form, followed by aecia. It was thought that telia which do occur on the host plant in nature are formed by the infection of mature leaves. Experiments on a large scale were therefore undertaken, and mature leaves of *Olea dioica* were sprayed with water and dusted with aeciospores on the undersurface. The inoculated plants were placed in moist chambers for 48 hours and later re-

moved to a greenhouse for observation. There were no visible signs of infection for 65 days, but yellow specks began to appear on the undersurface on the sixty-sixth day, gradually developing into circular orange-yellow spots. Emergence of the sporogenous tissue and development of the telia and teliospores were observed after eighty days. Repeated experiments with mature leaves in the manner just described have given confirmatory results.

It is manifest that the spore-forms which develop as a result of aeciospore infection are determined by the maturity of the leaves. On younger leaves the aeciospores assume the role of sporidia, without forming a promycelium, however, and later assume the role of normal aeciospores on older, mature leaves. To determine the stage at which the aeciospores produce telia, a small shoot on a potted plant was selected for experiment. Leaves developing in acropetal succession were infected with aeciospores in the usual manner and the plant kept in a moist chamber. The youngest leaves at the top formed infection spots in 6 days. These became hypertrophied, and pycnia and aecia were formed in due course. The oldest leaves at the base showed no signs of infection, even on the fiftieth day, but the leaves midway showed an interesting type of development. Some of them developed swollen infection spots with pycnia and aecia, but at places where no malformations had taken place, telia were observed after 30 days. On older leaves, however, telial formation took much more time; in fact, sometimes more than 100 days elapsed before the telia were manifest. The maturity of the host tissue therefore determined not only the time taken for the development of telia but also the nature of the spore-form that developed.

## Diagnosis of genus

The morphological facts now brought to light have necessitated an emendment of the generic and specific descriptions.

**Cystopsora** Butler, emend

Pycnia subepidermal, flask-shaped. Aecia subepidermal, cupulate, without peridia. Telia subepidermal, sporogenous hyphae emerging through stomata in fascicles; hyphae quite distinct, without lateral coalescence; teliospores developed in clusters from basal generative cell, formed at apex of sporogenous hypha; teliospores germinating without a rest period and directed toward the host; promycelium semi-internal, 2- or 4-celled; sporidia sessile; teliospores without germ pores.

Type species: *Cystopsora oleae* Butler on *Olea dioica* Roxb. at Khandala and Belgaum.

**Cystopsora oleae** Butler, emend

Pycnia amphigenous, distributed on hypertrophied patches and "witches' brooms," orange-yellow, flask-shaped, with ostiolar filaments. Aecia formed by displacing pycnia on hypertrophied patches, cupulate, yellow, erumpent; aeciospore chains converging toward center, peridia absent; aeciospores polyhedral, yellow, ovate-ellipsoid to fusiform, with reticulations on exospore and three distinct germ pores; aeciospores often developing within pycnia. Telia hypophyllous, sparsely distributed, orange-yellow, pulverulent, turning whitish following germination of teliospores, not causing hypertrophy on infection patches; sporogenous hyphae exserted through stomata in fascicles, distinctly separate; teliospores developed in clusters on discoid basal cell, 1-celled, pedicellate, spherical or ovate, deep orange-yellow, minutely verruculose; germina-

tion at maturity without a rest period, within sorus; promycelia directed toward the host, 2- or 4-celled, semi-internal; sporidia rather thick-walled, oval, sessile, binucleate.

Habitat on leaves and twigs of *Olea dioica* Roxb. at Khandala, Belgaum, Balehonnur (Mysore), and Bannerghatta (Bangalore).

## Summary

1. *Cystopsora oleae* has both 2- and 4-celled promycelia. The rust is an autoecious opsis form and causes hypertrophy and "witches' brooms" on young leaves and twigs. Infection patches on which telia are formed, however, are not malformed.

2. Teliospores germinate *in situ* without a rest period. Young teliospores are binucleate. Fusion takes place in mature spores. Promycelium is formed by the extension of the lower part of the teliospore, and the syncaryon moves into it and divides twice. If the promycelium is 4-celled the cells are uninucleate but are binucleate if it is 2-celled. The sporidia are always binucleate.

3. The binucleate condition persists, so far as it has been possible to determine, and it is presumed that the diplophase is initiated within the sporidium itself. The pycnium is thus borne on a diploid thallus, as all the associated mycelium has a pair of conjugate nuclei in each cell, although the pycniospores are uninucleate. Formation of aeciospores within the pycnia has been observed many times.

4. When aeciospores are sown on young leaves, they invariably give rise to pycnia, although they have never been observed to form promycelia and sporidia. When older leaves are infected, teliospore development takes place, but after a lapse of several weeks.

5. This rust thus presents certain remarkable characteristics not previously observed among other rusts and appears to be a form in a very unstable condition.

6. The diagnostic characters of the genus and species have been emended.

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# TRANSLOCATION OF THE REPRODUCTIVE STIMULUS IN SUGAR BEETS

MYRON STOUT

## Introduction

Sugar beets vary widely in their thermal requirements for reproductive development. Most of the economic varieties require prolonged exposure to cool temperatures (that is, extensive thermal induction) before they will produce seedstalks. They may be regarded as biennials. This general characteristic is due principally to the fact that generally in sugar-beet breeding the effort has been made to suppress the bolting tendency, since production of seedstalks is undesirable. There are non-economic varieties that require so little thermal induction that they will bolt, even when planted during the hot summer months in the vicinity of Salt Lake City, Utah. Such varieties may be classed as annuals. They have been found useful in certain types of experimental work.

All beet varieties, however, require long photoperiods before they will bolt normally. And all varieties may be kept in a vegetative condition indefinitely by growing them in an environment of short photoperiods and warm temperature. Furthermore, even though reproductive development has been initiated in an environment favorable to bolting, reversion to the vegetative type of growth may be effected by moving the plants to an environment of short photoperiods and warm temperature.

Annual beets that have started to bolt and then reverted to a vegetative condition have rosettes of leaves at the ends of the seedstalks. These terminal areas

react to photoperiods in the same way as do annual beets that have not bolted previously. Use of this fact has been made in some experiments here reported to obtain annual beets with growing points widely separated and hence more easily subjected to differences in light exposures.

The fact that the response of plants to photoperiod is localized rather than systemic was shown by GARNER and ALLARD (8) in 1925. Other workers have shown that the photoperiodic stimulus can be translocated to a part of the plant serving as receptor by defoliating this part and thereby inducing a flow of food into it.

The work reported here is mainly based on the photoperiodic response of beets; but the fact is recognized that, at least with biennial beets, thermal induction is an important factor. These two factors exert their influence on separate parts of the plant. KNOTT (12) and others have shown with various plants that the photoperiodic sensitivity is localized in the leaves. Unpublished studies by the writer have shown this to be the case in annual beets. CURTIS and CHANG (7) and CHROBOCZEK (6), working with celery and beets, respectively, showed that the thermal induction stimulus is perceived in the crown. The evidence that light and temperature are complementary factors encouraged the effort to conduct the present studies with the light factor alone.

Grafting as a means of studying photoperiodic response of plants has been employed by a number of workers (3, 4, 10, 11, 13, 14, 15, 17). CHOLODNY (5) has reviewed several of these papers. It has been shown that the substance or stimulus produced in a short-day plant as the result of exposure to short photoperiods is similar to that produced in a long-day plant by long photoperiods. MELCHERS (13) induced flowering in biennial plants of *Hyoscyamus niger* L. by grafting on them flowering scions of annual plants or of low-temperature treated biennials. NAYLOR (15) grafted annual and biennial varieties of sugar beets and grew both parts under long photoperiods, but since he did not provide the conditions necessary for translocation of food between the grafted parts, each section developed independently of the other. The annual part bolted while the biennial part remained vegetative.

The use of annual-biennial grafted sugar beets was thought to afford an excellent means for studying certain questions that naturally arise in considering the thermal and photoperiodic factors in reproductive development in relation to the kind of substance or stimulus produced by each. Do annuals require only one kind of substance or stimulus, while biennials require two kinds? Or, do both of these environmental factors contribute toward the production of one and the same kind of substance or stimulus? Further information concerning the answers to these questions should aid in the solution of the internal causes of reproductive development. Considerable information has accumulated concerning the environmental factors responsible for reproductive development in plants, but as yet little or nothing is known about the mechanism of the stimulus or the substance or substances involved.

### Material and methods

A variety of annual beets, usually referred to as the Munerati annual, S.L. 2240 or S.L. 841, was used in the early translocation studies. In later studies with grafted beets the annual component was a hybrid between the Munerati annual and a curly-top-resistant biennial variety. This hybrid, S.L. 2850, was

found to be definitely annual in character and had the advantage of better vigor and some curly-top resistance. S.L. 234, a nonbolting curly-top-resistant variety, was used as the biennial component in all the grafting experiments.

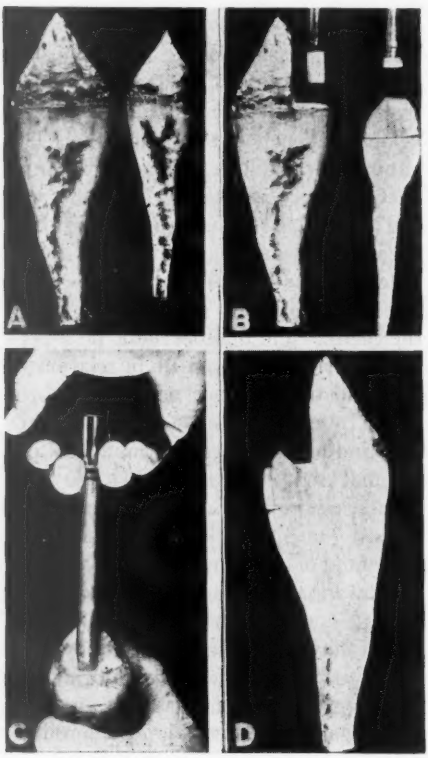


FIG. 1.—Corkborer technique used to graft sugar beets. *A*, beet used as stock at left, that used as source of scion at right. *B*, cylinders of tissue removed from both stock and scion. *C*, heavy-walled metal tubing, reamed out to fit conical shoulder of scion, used to force scion from corkborer into stock. *D*, longisection of grafted beet.

Annual beets having more than one growing point were obtained by two methods, both of which gave plants that reacted similarly. In the first method the leaves and petioles of vegetatively growing plants were trimmed off and the tops split to a point about 2 inches below the crown. A pebble was placed in the crotch and the beets were potted and grown in a warm, short-photoperiod environment for about 2 months, or until a single well-developed rosette of leaves was obtained on each segment. In the second method the plants were allowed to start to form seedstalks, the tops of the seedstalks were pinched off to induce lateral branching, then the beets were placed in a warm, short-photoperiod environment until single, well-developed rosettes of leaves were formed on two or more branches. The latter method required more time but produced plants that were more easily covered.

Heavy black cloth bags covered with white sheeting were used to cover parts of the plants to be kept darkened. The bags were kept from collapsing by inside wire frames and were closed with drawstrings. The exposed parts were given 17- to 18-hour photoperiods or continuous illumination. Supplemental illumination intensity was 25–50 foot-candles. In preliminary experiments it was found that the tips of seedstalks growing in the dark turned black and died after several weeks' treatment. An exposure of the covered part of the plant to about 2 hours of sunlight each week prevented this trouble. In all subsequent tests the plants were given this prophylactic treatment.

GRAFTING TECHNIQUE.—Several attempts were made to graft beets so that the scion would become entirely dependent upon the stock for mineral nutrients. The only method that proved satisfactory was based on one described by Goss (9) for inoculating potatoes with spindle-tuber disease. Use of that method for beet work was suggested to the writer by C. W. BENNETT, who had used it in curly-top studies at Riverside, California.

As used in the present work, the method is illustrated in figure 1 and may be described as follows: A beet about $1\frac{1}{2}$–$2\frac{1}{2}$ inches in diameter was used for the stock. The leaves were trimmed closely and a block of the crown tissue cut away to make a flat shoulder on one side, at about the level of the lowest leaf scar. From this shoulder, and extending down to a parallel incision about $\frac{3}{4}$ inches below, a cylinder of beet tissue about 11 mm. in diameter was removed by means of a corkborer.

A smaller beet was used for the scion. It was trimmed to remove the leaves and some of the crown tissue but without injury to the apical bud. A transverse incision was made in the shoulder of the beet and well past the center of the crown. This incision was at such distance from the top of the crown as to make the cylindrical scion the desired length to fit the space in the stock beet. The scion cylinder, including the apical bud, was cut by a corkborer about 1 mm. larger in diameter than the one used to make the space in the stock.

The scion was then forced from the corkborer into the stock by means of a heavy-walled, metal tube slightly smaller in diameter than the corkborer. The end of this tube was reamed out to fit the conical top of the scion, permitting pushing the scion without excessive injury. The scion was so inserted that the upper edge of its cylindrical surface was flush with or slightly above the flat surface of the stock. The cut surfaces, with the exception of the apices of the scion and stock, were then covered with melted grafting wax. Beets so grafted were kept in a humid chamber for about 2 weeks before they were transplanted to pots.

The grafted beets were grown in a warm, short-photoperiod environment for several months to reverse any thermal induction they had received. It was found that if the beets were taken from the field in the fall, when the crown tissue was firm, 75–90% of the scions grafted by this method grew. The crown tissue of greenhouse-grown beets was not firm enough to work well with the method.

## Experimental results

### Annual beets

Seven split annual beets were placed under differential conditions of illumination February 3, 1938. One shoot of each plant was exposed to 17-hour photoperiods; the opposite shoots of three plants were kept in continuous darkness; while the opposite shoots of the other four plants were exposed to 7-hour photoperiods. From 3 to 4 weeks were required to induce bolting in the 17-hour exposed shoots. Of the three that had one shoot kept in darkness, one darkened shoot bolted March 10, another April 1, and the third died before April 1. Of the four plants that had shoots exposed to 7-hour photoperiods, one died and the other three shoots remained vegetative.

In another test, one shoot of each of six split annual beets was exposed to 17-hour photoperiods, beginning April 4, 1938. The opposite shoots of three plants were kept in continuous darkness, while the opposite shoots of the other three plants were exposed to 4-hour photoperiods. The 17-hour exposed shoots bolted about April 25. The continuously covered shoots developed as follows: one bolted May 25, another June 6, and the third developed an elongated crown but did not form a typical seedstalk; all the three shoots exposed to 4-hour photoperiods remained vegetative.

In the previously described tests with split annual beets, a period of about 3–4

weeks was required to induce bolting in the long-day exposed part and about a month of additional time to induce bolting in the attached part kept in the dark. In tests with beets that had bolted and then reverted to a vegetative condition, the time required to induce bolting in continuously exposed parts was only about 2 weeks, and bolting of the part kept in the dark followed that of the continuously illuminated part much more closely. In some instances the part exposed to continuous illumination and that kept in continuous darkness bolted almost simultaneously. Only one of the latter tests will be described in detail.

FIG. 2.—Annual beet with three shoots that were exposed to photoperiods, after May 28, 1941, as follows: left, 7 hours; center, continuous darkness; right, continuous illumination. Shoot at right bolted June 5; one in center had seedstalk measuring 3 inches June 18; one at left remained vegetative. Photographed August 9, 1941.

Seven annual plants that had bolted and then reverted to a vegetative condition were given differential photoperiodic treatments beginning May 28, 1941. Two plants had three shoots each, and each shoot was given a different treatment: continuous illumination, 7-hour photoperiods, and continuous darkness. Four plants having two shoots each had one shoot exposed to continuous illumination and the other to continuous darkness. The other plant, having two shoots, had one exposed to continuous illumination and the other to 7-hour photoperiods. In all, there were seven shoots exposed to continuous illumination, three to 7-hour photoperiods, and six to continuous darkness. Seedstalks had started to develop on all shoots given continuous illumination by June 5. All those kept in continuous darkness had seedstalks measuring $1\frac{1}{2}$–$5\frac{1}{2}$ inches in length by June 18, indicating that bolting had started in some about a week earlier. All the growing points exposed to 7-hour photoperiods remained vegetative. The typical effect of each treatment can be illustrated in one of the plants that had three shoots, each treated differently. The plant shown in figure 2 indicates that the reproductive stimulus translocated from the continuously illuminated part (right) to that kept in darkness (center) was not appreciably decreased or inhibited by any negative stimulus from the attached vegetative part that received 7-hour photoperiods (left).

ANNUAL-BIENNIAL GRAFTED BEETS

Annual (variety S.L. 2850) and biennial (variety S.L. 234) beets were taken from an overwintering seed-field in St. George, Utah, March 4, 1943. They were grafted March 10 and kept in a warm, humid chamber for about 2 weeks and then potted and grown in a warm, short-photoperiod environment (to reverse thermal induction [16] in the biennial variety) until August 13, 1943. During this period the beets were frequently trimmed of all but one vegetative shoot on each stock or scion. After August 13, one part of each plant was given continuous illumination and the other part kept in darkness.

Seven plants, in which the annual stock was kept in darkness, were started in the test. Four of the plants died. The three that lived are shown in figure 3*A*. Both parts of each plant remained vegetative, with one exception. The annual part of the large plant at the left started seedstalk formation early in the test (September 27), but subsequent elongation of the seedstalk did not follow, and when the photograph was taken (December 13) the growth had reverted to a vegetative condition. It is probable that small leaflets that developed on the crown of the annual part below the cover were not removed frequently enough at first and may have been responsible for initiation of the seedstalk, rather than any stimulus translocated from the exposed biennial part. The fact that the seedstalk reverted to a vegetative rosette after being initiated further supports this explanation.

Eight grafted plants, in which the annual scion was kept in darkness, are shown in figure 3*B*. With one exception, both parts of each plant remained vegetative throughout the test. The plant at the upper left of figure 3*B* grew vegetatively until November 19 (98 days), when a seedstalk started to develop on the exposed biennial stock. The cover on the annual scion was removed and a seedstalk had also started to develop there. This was the only instance in which a light-exposed biennial part of

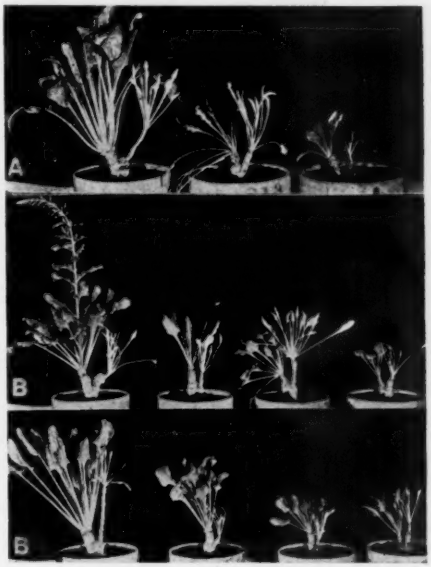


FIG. 3.—Annual-biennial grafted beets in which biennial part (left side of each beet) was exposed to continuous illumination while annual part was kept in darkness. *A*, biennial part as scion; *B*, biennial part as stock. Differential lighting started August 13; photographed December 13, 1943.

the grafted plants bolted, and it was probably caused by cooler temperatures of the greenhouse during the last part of October and the month of November. The fact that only one of the biennial beets in this group of plants bolted is probably due to genetic variation.

Four annual-biennial grafted beets, in which the biennial scion was kept in continuous darkness, are shown in figure 4*A*. The continuously illuminated annual stocks had all started to bolt by Septem-

ber 13. The two biennial scions on the left of the figure bolted, one on October 5 and the other on December 8. Lack of vigor of the other two biennial scions may have been responsible for their remaining vegetative.

bolted (from left to right) September 27, October 5, and September 27. Three to six weeks' exposure to continuous illumination was evidently required to produce the stimulus or substance in the leaves of the annual, translocate it to the apical

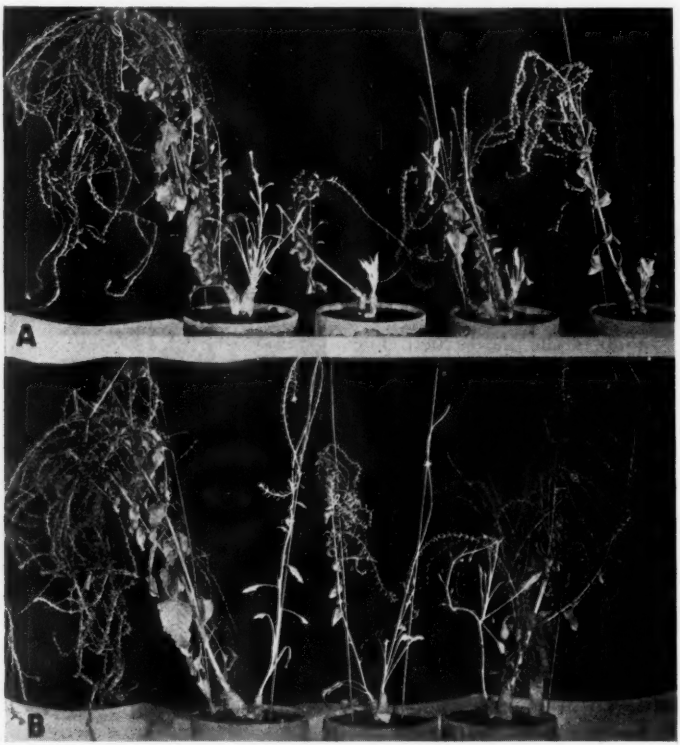


FIG. 4.—Annual-biennial grafted beets in which annual part was exposed to continuous illumination while biennial part was kept in darkness. *A*, annual part as stock; *B*, annual part as scion. Differential lighting started August 13; photographed December 13, 1943.

Three grafted plants, in which the biennial stock was kept in continuous darkness, are shown in figure 4*B*. The continuously illuminated annual scions bolted on the following dates: From left to right, September 6, 27, and 13. The continuously darkened biennial stocks bud, and induce formation of a seed-stalk. It is also evident that only 8–21 days of additional time was necessary to induce bolting in the continuously darkened biennial parts of the same grafted plants. This evidence of effective similarity regarding requirements for reproduc-

tive development in annual and biennial beets will be discussed more fully later.

## Discussion

Production of a food deficit in one part of a plant by defoliation or by keeping it in darkness has proved a useful tool in the study of the translocation of substances other than plant food. The work of BENNETT (1) on the translocation of viruses showed that curly-top virus was rapidly translocated from a diseased to a healthy shoot of a sugar beet when the healthy shoot was defoliated or kept in darkness. In some instances 24 hours was sufficient to translocate the virus to the healthy shoot. In this case a short period of translocation would supply the small amount of virus necessary to initiate the disease. On the other hand, to cause bolting, a continuous translocation of reproductive stimulus or substance probably is required.

When annual beets were split similarly to those used by BENNETT, 3–5 weeks of darkness were required for evidence of bolting in the darkened side after the illuminated side had started to bolt. In these instances the two growing points were separated by a considerable reservoir of storage tissue, upon which the darkened shoot probably drew for food as well as from the shoot exposed to long photoperiods. Because the food for the darkened shoot presumably came from these two sources, accumulation in this shoot of an adequate amount of the reproductive substance was delayed. There was only about half as much lag in bolting response between the exposed and the darkened shoots in the beets that had bolted and then reverted to the vegetative state before being given the differential illumination. The growing points in these beets, even though farther apart than in the split beets, were separated by stem tissue rather than by storage tissue. The difference in time required between the two types of plants to develop seedstalks is probably further evidence that a synthesized, flower-inducing substance, translocated with the elaborated food, is involved in reproductive development. The negative results of NAYLOR (15), who grafted annual and biennial varieties of sugar beets, also show that translocation of the reproductive substance follows that of synthesized food. Because he created no food deficit in the biennial, there was no transfer of reproductive substance from the annual part.

The darkened shoot of annual beets that had three shoots, one of which was kept vegetative (fig. 2), bolted as rapidly as darkened shoots on plants that had no vegetative shoots, indicating that vegetatively growing plants do not produce any substance or stimulus antagonistic to reproductive development but merely lack the substance or stimulus in sufficient amounts to cause it. This evidence confirms the conclusions of CAJLACHJAN (2) in this regard.

Several workers have shown that the substance or stimulus produced in long-day plants under long photoperiods is in effect the same as that produced in short-day plants under short photoperiods. They used defoliation of the receptor to induce translocation of the flowering stimulus. MELCHERS (13) showed that biennial plants of *Hyoscyamus niger* L. could be forced to initiate reproductive development by grafting to them either flowering scions of annuals or scions from cold-treated biennial plants. He postulated that the substance produced by the annuals and biennials was the same, but that a gene in the biennial introduces an inhibitory effect that is eliminated by treatment at low temperature. The available data indicate that the

flower-inducing substance or stimulus produced in annual beets under the influence of long photoperiods alone is in effect similar to that produced in biennial beets as a result of the combined effect of cool-temperature exposure and long photoperiods.

If the flower-inducing substance is the same in annual and biennial beets, it would appear that the leaves of the annual can synthesize an adequate amount of the substance under long photoperiods and that the leaves of the biennial cannot, except possibly under continuous illumination of high intensity. Another possibility is that an activator or precursor is genetically supplied to the annual, while in the biennial it is formed as a result of cool-temperature exposure.

The fact that the two environmental factors, light and temperature, affecting respectively the leaves and the crown, produce a similar effect suggests the possibility of obtaining evidence on the biochemical basis of reproductive development. Possibly the same flower-inducing substance will be discovered to be formed in beet leaves and crowns.

## Summary

1. Separate shoots of annual beets having more than one shoot were subjected to differential photoperiods as follows: long days or continuous illumination, short days, and continuous darkness. The parts exposed to long days or continuous illumination developed seedstalks. The parts exposed to short days, even though connected to parts exposed to long days or continuous illumination, remained vegetative. The parts kept in continuous darkness and connected to parts exposed to long days or continuous illumination developed seedstalks, even when they were also connected to short-day exposed parts that remained vegetative.

2. These results indicate that substances conducive to reproductive development may be translocated with the carbohydrates. Exposure of a part to short photoperiods produced sufficient carbohydrates for that part, and hence none was translocated to it from the long-day part. Keeping a part in continuous darkness made that part dependent on the long-day exposed part, and hence food, and with it reproductive-inducing substances, were translocated to the darkened part.

3. Continuously illuminated biennial parts of annual-biennial grafted beets failed to develop seedstalks when they were not also subjected to cool-temperature exposure. Neither did the continuously darkened annual parts of the same beets develop seedstalks.

4. About one month was required to initiate seedstalks in continuously illuminated annual parts of annual-biennial grafted beets. The continuously darkened biennial parts of most of the same beets also developed seedstalks during the following month.

5. These results indicate that the biennial is not able to synthesize reproductive-inducing substances as a result of photoperiod alone, as annuals do, but also requires cool-temperature exposure. The reproductive-inducing substances produced by biennials as a result of thermal and photoperiodic induction, and those produced in annuals as a result of photoperiodic induction alone, appear to be in effect similar.

6. A simple and effective method of grafting such material is described.

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## CICATRIZATION IN LEAVES OF BRYOPHYLLUM CALYCINUM

WALTER B. WELCH

### Introduction

The leaves of *Bryophyllum calycinum* Salish. are well known for their ability to retain moisture after having been detached from the plant. During an investigation on the water relations of leaves during severe drying (**7**), small corky layers were found to have formed over the end of the petiole when the leaves were allowed to dry in the open air. These same corky layers were seen at any injured place on the leaf. When the leaves were transferred from their drying trays to the pan of a balance for weighing, the corky layers would drop from the leaf.

SAMUEL (**5**) states that in a moist atmosphere with a good supply of water to the leaf all injured tissues will be abscised and that in a dry atmosphere with a poor supply of water to the roots abcission will not occur. He states further that in a moderately dry atmosphere abscission will or will not occur, depending upon whether the meristem arises some distance within the leaf lamina or near the edge of the injury. PRIESTLY and WOFFENDEN (**4**) found in potato tubers that,

if the injured tubers were exposed to moist atmosphere, the healing of wounds was more rapid and continuous; and if exposed to dry atmosphere, especially in sunlight, the healing was slower and not continuous. BLACKMAN and MATTHAEI (1) found that "the factor of excessive drying does not come in."

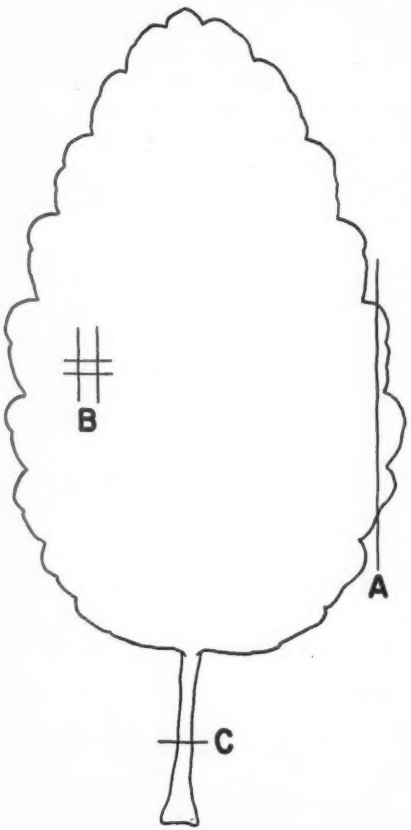



FIG. 1.—Outline of leaf of *Bryophyllum calycinum* showing positions of wounds. *A*, wound along margin of leaf; *B*, cross-hatch wound of center; *C*, wound of petiole.

Since the corky layers did fall from the leaf with the appearance of true abscission, an investigation was undertaken to determine whether there was an active meristem and abscission layer of the cicatrice formed in the leaf. WYLIE (8) has found that the thicker the leaf the wider the cicatrice, except in succulents or semi-succulents. There seems to be no constant relation for all leaves. The leaves of *Bryophyllum* might certainly be called succulent, or they might be considered xerophytic—if MAXIMOV'S (2) criteria were used. The leaves of *Bryophyllum* will produce small plantlets on the margin at the notches, even after the leaf has wilted, if the leaf is removed from the plant in a turgid condition. If the leaf is allowed to wilt as the plant dries up and is then removed, it will produce few if any plantlets.

## Material and methods

The material of *Bryophyllum calycinum* used in this investigation was that which is known as the Chicago variety (3). The plants were grown in the greenhouse at the University of Chicago, and also in the greenhouse of Southern Illinois Normal University, under ordinary greenhouse conditions until they had produced at least eight sets of leaves. At the fourth and fifth nodes the leaves no longer increase in size. These were considered mature and used in this investigation.

Two series of leaves were established. In the first the leaves were detached from the fourth and fifth nodes of vigorously growing plants. These were placed on screen-wire trays and allowed to dry at room temperature varying from 50° F. to 85° F. and at laboratory humidity from 50% to 85%. In the other series the leaves remained attached to the plants that were growing under greenhouse conditions. The temperature of the greenhouse was never over 85° F. or under 55° F. The relative humidity was

rarely as much as 90% and never under 60%.

The series of leaves allowed to dry in the laboratory was divided into three sets. One set was cut along the margin with a sharp razor blade (fig. 1*A;* hereafter shown as *A* in all figures). This cut was made through all the tissues and the margin removed and discarded. Another set of leaves was cut with crosshatch lines (fig. 1*B;* hereafter shown as *B* in all figures). This was about halfway from the midrib to the margin and at about the widest portion of the leaf. These leaves were cut through the upper epidermis and into the mesophyll but not through the lower epidermis. The third set had the petiole cut off about halfway from the point of attachment to the base of the blade (fig. 1*C;* hereafter shown as *C* in figures).

Leaves of the second series were left attached to the plant and only those from the fourth and fifth nodes were wounded. The wounds were made as in the first series, except at the petiole. It was impossible to make a cut more than halfway across this organ and have it remain on the plant, and it was found more practical to cut through about two-fifths of the tissues, including one of the lateral bundles.

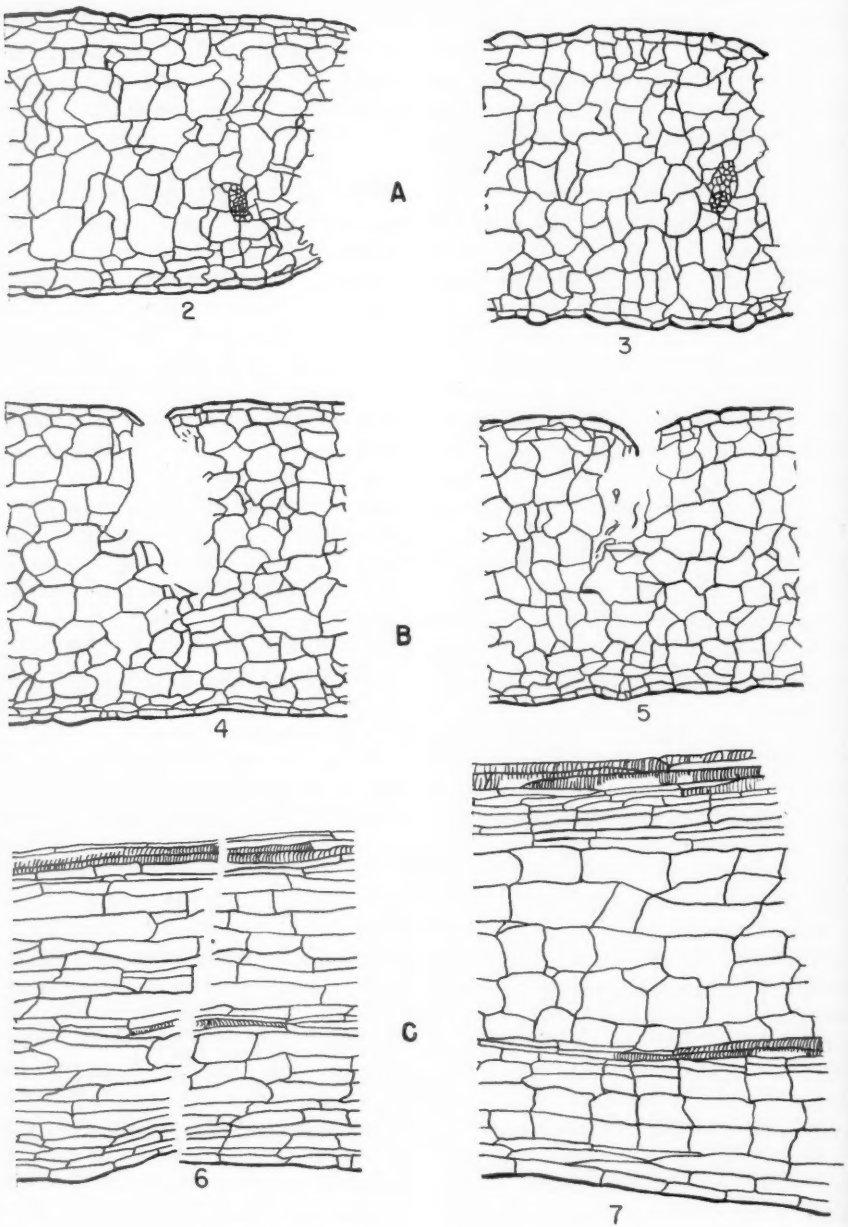
In each series all leaves were wounded on the same day and at as nearly the same time as possible. The first collection was made at once, and then every 4 hours for the next 48 hours. After that, collections were made every day for 2 weeks. After 2 weeks a collection was made every 4 days for the next 9 weeks.

The specimens were fixed in a solution of 6.5 cc. formalin, 2.5 cc. glacial acetic acid, and 50% alcohol to make 100 cc.; mounted in paraffin and sectioned at 10 μ. The drawings were made with the aid of a Promi projector and all were magnified twenty-eight times. In the sections of the leaf blade no attempt was made to include or exclude either the veins or the stomata.

## Discussion

The leaf of *Bryophyllum calycinum* has some of the characteristics of a succulent, in that the ratio of internally to externally exposed surface is comparatively low (**6**). The relative number of stomata is also small (**7**). In the illustrations the intercellular spaces are indicated only by the curvature of the cell walls as they jut into the intercellular space. In this leaf there is little to distinguish the types of mesophyll cells. After the leaf has started to dry out, the palisade and spongy cells are almost indistinguishable. The cuticle is not as thick as might be expected on a succulent leaf. In the drawings it may even be exaggerated, owing to the width of the line on the surface of the epidermis. There was no attempt to show the contents in any of the cells.

When the first samples were taken they were plunged immediately into the fixative. A little collapse will be noticed in the leaf section taken from the margin *A* (fig. 2). There is little difference between the leaf that was attached to the plant (fig. 2) and the one that was removed to dry in the open air (fig. 3). The sections taken from the center of the leaf (figs. 4, 5) show more collapse. This may have been due to the razor blade not being very sharp. In the sections of the petiole (figs. 6, 7) where the tissues were more rigid the cut was clean and no collapse was seen. The lack of differentiation in the mesophyll is shown in the drawings. These would be considered the "normal" sections. The wounds were made a matter of minutes before being plunged into the killing fluid and there-




FIGS. 2–7.—Sections of leaves fixed immediately after wound was made, or at 0 hours: *A*, marginal wounds; *B*, cross-hatch wounds; *C*, wounds of petioles. Fig. 2, attached leaf. Fig. 3, removed leaf. Fig. 4, attached leaf. Fig. 5, removed leaf. Fig. 6, attached leaf. Fig. 7, removed leaf.

fore the response is none or very little. There is only slight evidence of any destruction of the cells other than that done by the razor.

Four hours after the wounds were made, some changes were evident in the cells along the margin of the wound *A*. In the sections *A* (figs. 8, 9) the epidermis curls over the wound, and in some cases the upper epidermis is almost touching the lower. A much greater area has started to dry out in the leaf that was attached to the plant (fig. 8) than in the leaf that was removed (fig. 9). In the attached leaf there are some twelve rows of cells that have started to collapse, while in the leaf that was removed there are only eight rows. The curling of the epidermis and the collapse of the cells adjacent to the wound may be what PRIESTLY and WOFFENDEN (4) refer to as an effective block to the transpiration from a wound surface.

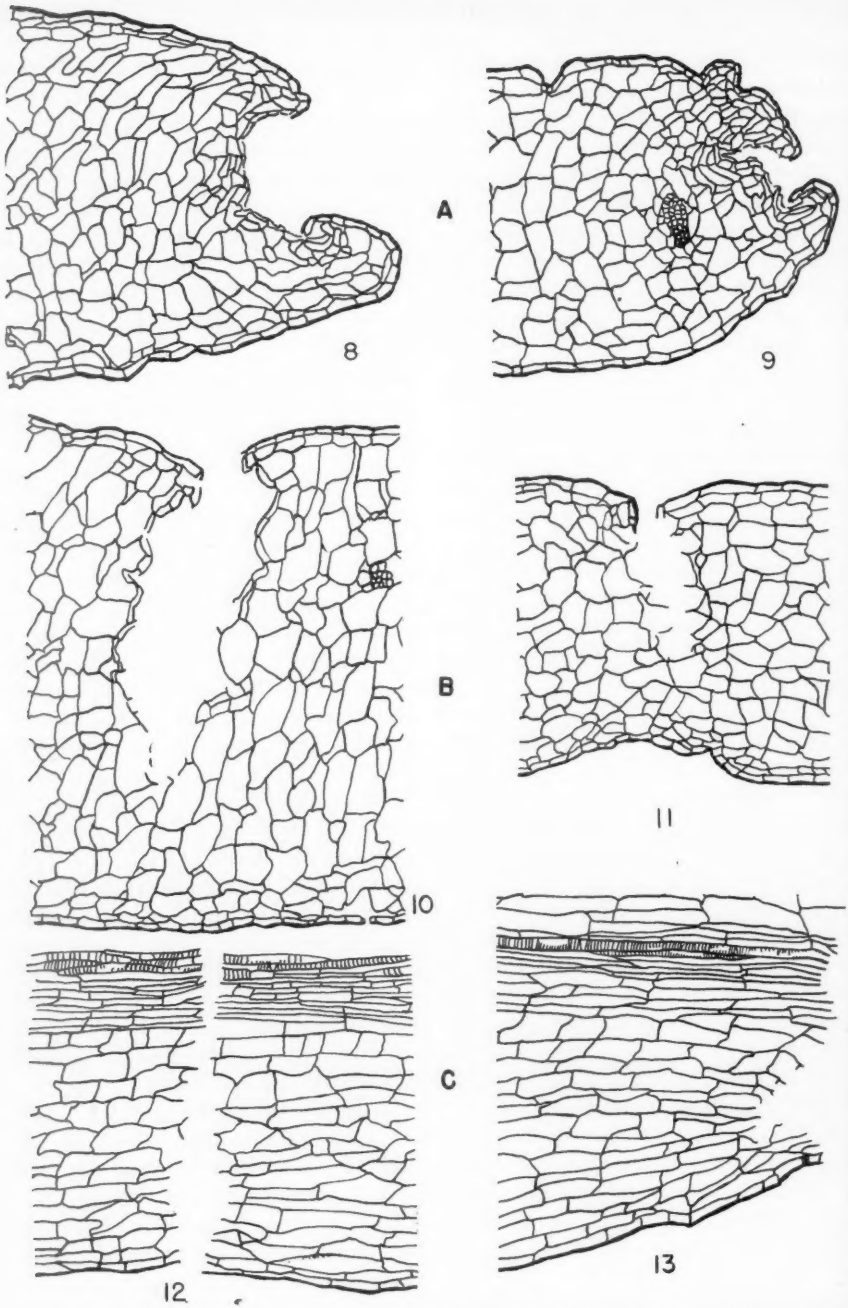
Sections *B* (figs. 10, 11) show much less of the collapse due to the drying of the cells than sections *A*. Here the wound was through the upper epidermis and the upper part of the mesophyll. The evaporation from this wound must have been reduced to a minimum, for very little moisture was lost through it. Here also the area of collapse seems to be greater in the leaf that was attached (fig. 10) than in the one that was removed (fig. 11). The difference in the sizes of the leaf is not due to one drying out more than the other. The leaves did vary a great deal in thickness.

In the sections of the petiole at *C* (figs. 12, 13) there is very little change in the leaf that was attached as compared with the section that was made 4 hours before. In this section the wound was made only two-fifths of the way across the petiole, so that it could remain on the plant (fig. 12). Very little evaporation has taken place, since little of the wound area was exposed. In the section where the end was cut off the petiole (fig. 13) the whole area was exposed and therefore evaporation was great. Almost as much evaporation takes place as in the sections *A*. The epidermis starts to curl over the cut tissues, and the only difference from sections *A* is the vascular bundle that keeps the organ rigid.

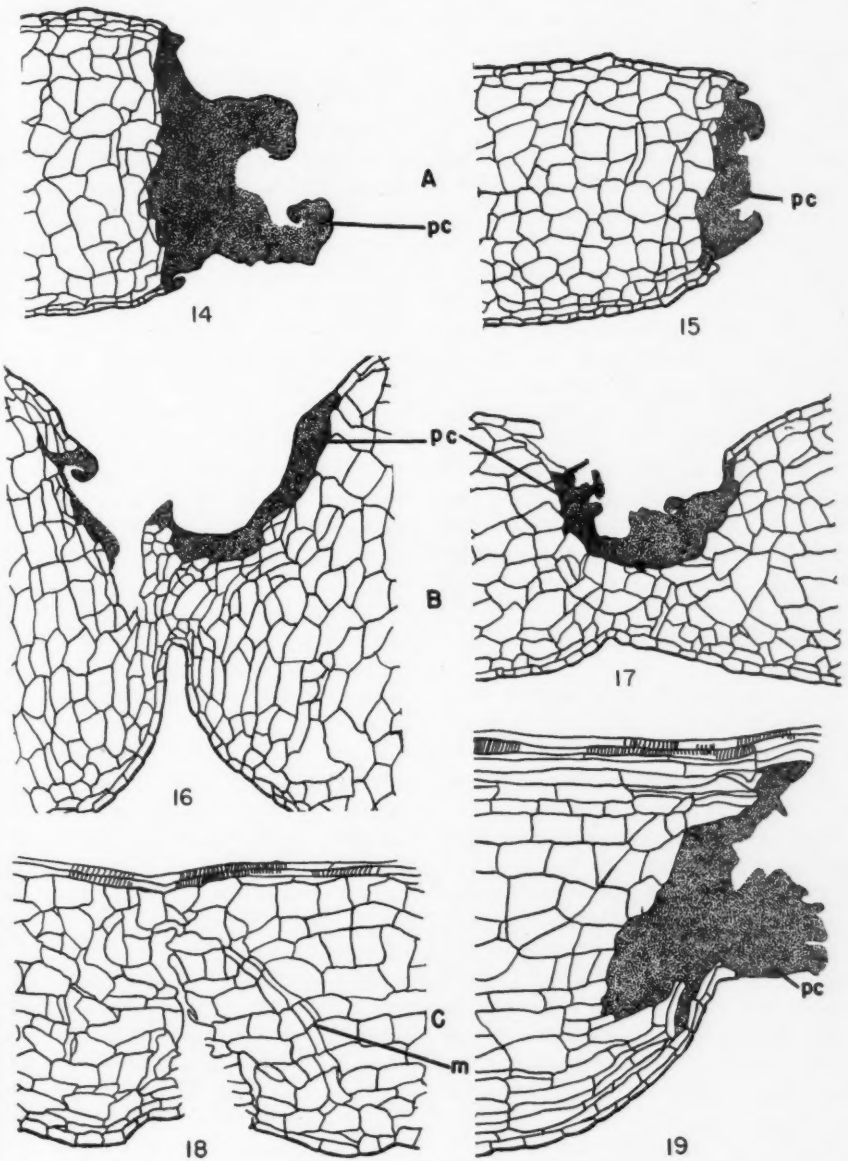
After 3 days many of the cells that showed collapse in the first 4 hours are dead, and so decomposed as to be unrecognizable. This layer acts as a further block to the evaporation from the living cells below. In the sections *A* (figs. 14, 15) these areas are shown by the heavy shading. In both figures the cells next to the dead decomposed tissues seem to be as turgid as any others of the leaf. It is apparent that the leaf that was attached (fig. 14) lost more tissue by collapse owing to drying than did the leaf that had been removed. Again it might be pointed out that the leaves that were removed from the plant had no contact with moisture other than that of the air.

In the sections of the cross-hatch wound in the center of the leaf (*B*), the leaf that was attached (fig. 16) shows much greater collapse than the one that was removed (fig. 17). Relatively there are a greater number of dead cells in the leaf that was removed than in the one that was attached. Here also the living cells next to the dead tissue seem to be as turgid as any of the others.

In the sections of the petiole at *C*, the leaf removed from the plant (fig. 19) shows a much larger area of the dead decomposed cells than does the leaf that remained on the plant (fig. 18). The leaf that was removed also shows a curling of the epidermis that will partly cover the decomposed cells. This follows the expected procedure, since there was a large




FIGS. 8–13.—Sections of leaves 4 hours after wounds were made: *A*, marginal wounds; *B*, cross-hatch wounds; *C*, wounds of petioles. Fig. 8, attached leaf. Fig. 9, removed leaf. Fig. 10, attached leaf. Fig. 11 removed leaf. Fig. 12, attached leaf. Fig. 13, removed leaf.




FIGS. 14–19.—Sections of leaves 3 days after wounds were made: *A*, marginal wounds; *B*, cross-hatch wounds; *C*, wounds of petioles. Fig. 14, attached leaf. Fig. 15, removed leaf. Fig. 16, attached leaf. Fig. 17, removed leaf. Fig. 18, attached leaf. Fig. 19, removed leaf. *m*, meristem; *pc*, pseudocicatrice.

area of collapsed tissue in the petiole at 4 hours. There is no decomposition of the cells in the petiole of the leaf that was attached to the plant, and there is very little collapse of the tissues along the wound, but a new structure is developing the meristem that will later form the cicatrice (fig. 18*m*). In following WYLIE'S (8) nomenclature, the meristem will be called the meristem, and the tissues produced by it (the cicatrice and those between the cicatrice and the wound) the pseudocicatrice. In the pseudocicatrice there appears, later in the development of the cicatrice, some cells that lose their cell contents and are cut off from the living tissues but do not decompose and lose their identity. In the section (fig. 18) of the petiole that is attached to the plant there is a meristem formed before any of the decomposed tissue is apparent.

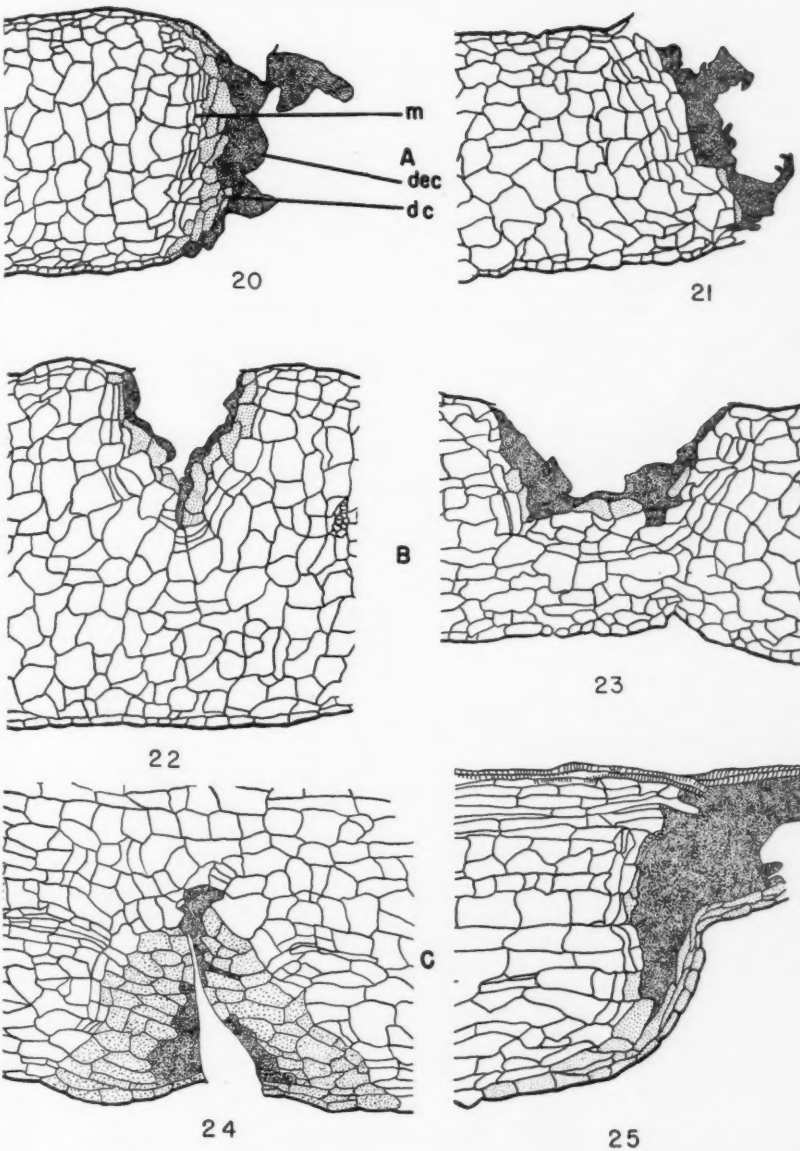
The meristem divides to form the cicatrice and cuts off a wall of living cells (fig. 20) back of the decomposed cells of the pseudocicatrice. The wall of living cells may be only one to seven cells wide. The pseudocicatrice has two types of cells, the decomposed cells of the first collapse and now the dead cells cut from the rest of the tissues by the cicatrice. The dead cells of the pseudocicatrice are easily distinguished and are without cell contents. The decomposed cells of the pseudocicatrice take a very dark stain characteristic of lignified tissue, while dead cells take a stain characteristic of suberized tissue. The outer cells of the cicatrice soon show the suberin reaction; in some cases the outer wall shows suberin as early as 2 days after they have been formed. The dead cells are thus cut off from water and food, and (fig. 20) become part of the pseudocicatrice.

After 4 days the meristem has up to four cell divisions. The leaf that remained attached has more cells cut off in the pseudocicatrice (lightly shaded areas of figs. 21, 23, 25) and more divisions of the meristem.

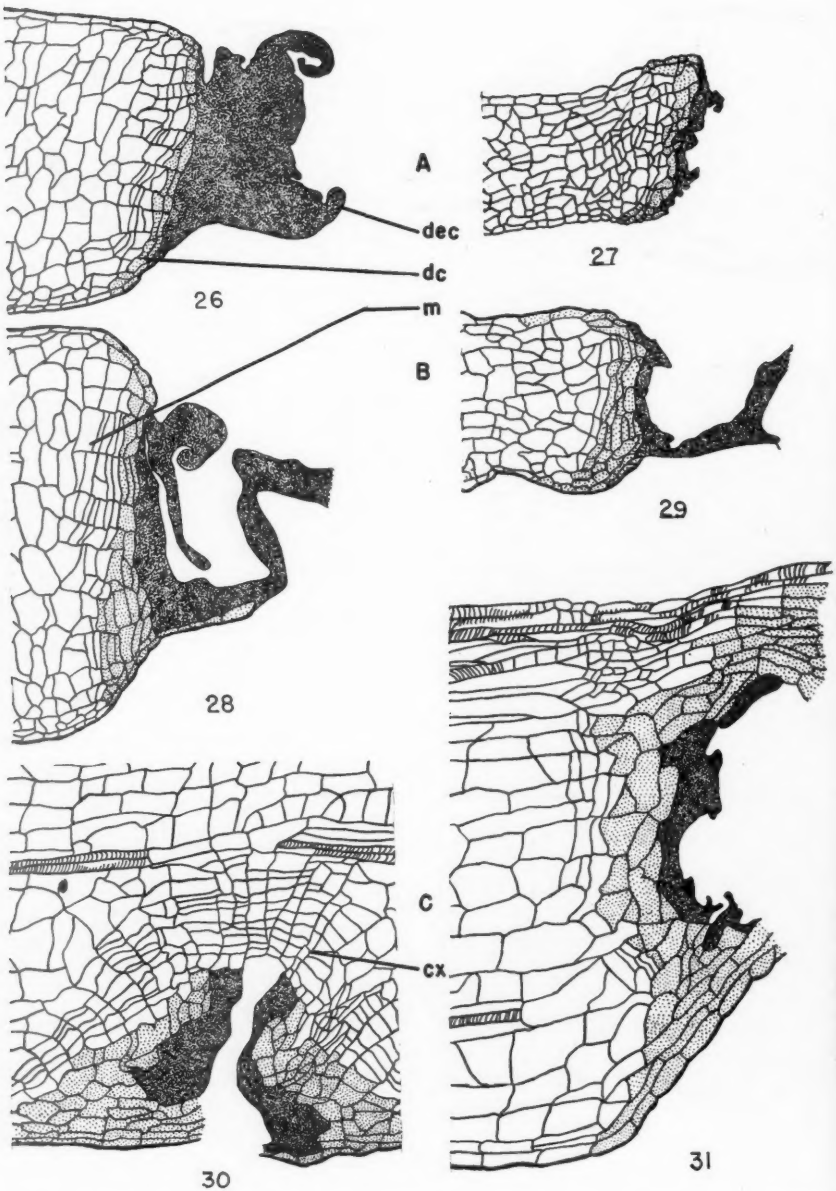
After 6 days there is greater meristematic activity in the attached leaf than in the detached one (figs. 26, 28, 30, 27, 29, 31). Some of the cicatrice cells produced by the meristem have become suberized and no longer have cell contents. This is shown in the figures by the light shading. The cells of the bundle sheath of the petiole (fig. 31) have become meristematic and have grown in between the ends of the spiral elements of the xylem. In detached leaves the effect of drying out is evident. The cells have decreased in size and there is a swollen area where the meristem is active (figs. 27, 29). This swollen area was apparent in all detached leaves, no matter where the wound was made. It is not entirely due to the rigidity of the cicatrice and pseudocicatrice, for sometimes it is some distance behind the meristem, and a sunken area appears between the meristem and the cicatrice. There is apparently a water-storage tissue developed just back of the meristem, this tissue becoming more apparent as the drying continues (figs. 33, 35).

At the end of 6 weeks the cicatrice and many cells of the meristem have become suberized and are dead. There is some indication of lignin in the dead cells of the pseudocicatrice. The meristem has invaded the epidermis and produced a cicatrice sixteen cells away from the wound in some cases (figs. 32, 33, 34, 35). The sections of the detached leaf show the cells almost crushed (figs. 33, 35). In another week the whole leaf (except the petiole) is brittle and shatters at touch.

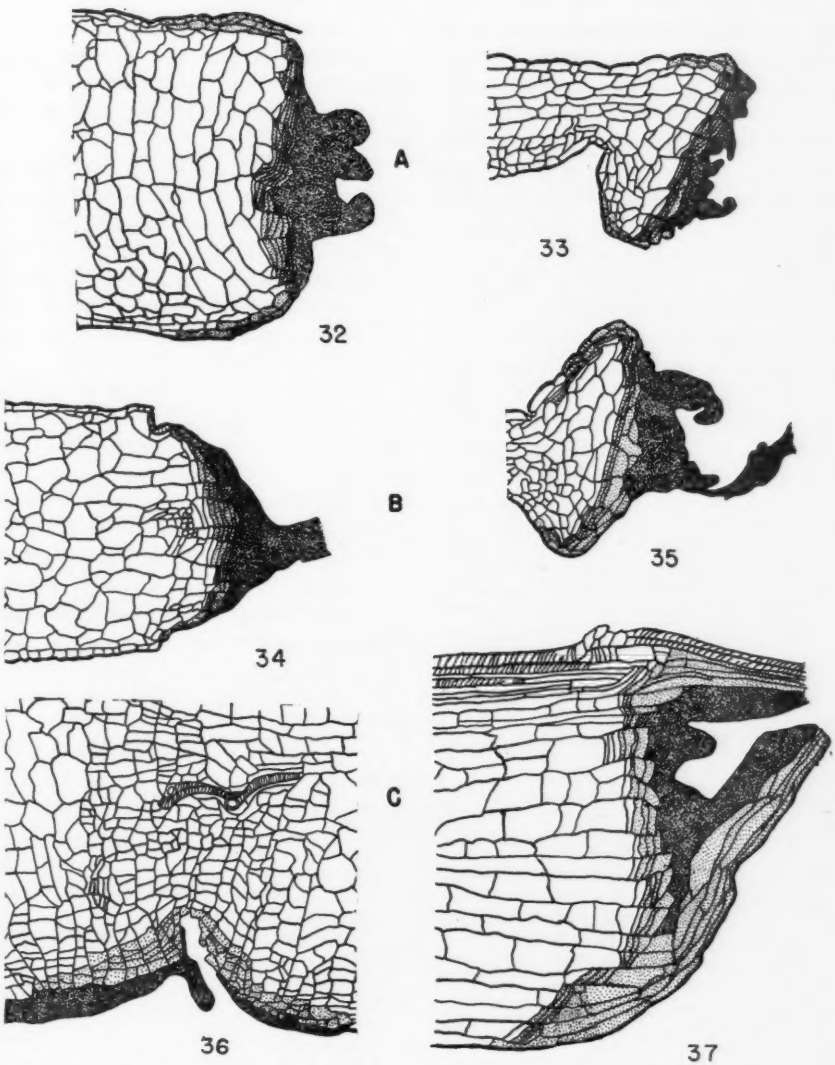
There is no evidence of any abscission of the pseudocicatrice or of the cicatrice. In fact, there is some evidence that the




FIGS. 20–25.—Sections of leaves 4 days after wounds were made: *A*, marginal wounds; *B*, cross-hatch wounds; *C*, wounds of petioles. Fig. 20, attached leaf. Fig. 21, removed leaf. Fig. 22, attached leaf. Fig. 23, removed leaf. Fig. 24, attached leaf. Fig. 25, removed leaf. *dc*, dead cells of pseudocicatrice; *dec*, decomposed cells of pseudocicatrice; *m*, meristem.




FIGS. 26–31.—Sections of leaves 6 days after wounds were made: *A*, marginal wounds; *B*, cross-hatch wounds; *C*, wounds of petioles. Fig. 26, attached leaf. Fig. 27, removed leaf. Fig. 28, attached leaf. Fig. 29, removed leaf. Fig. 30, attached leaf. Fig. 31, removed leaf. *cx*, cicatrice; *dc*, dead cells of pseudocicatrice; dec, decomposed cells of pseudocicatrice; *m*, meristem.




FIGS. 32–37.—Sections of leaves 10 weeks after wounds were made: *A*, marginal wounds; *B*, cross-hatch wounds; *C*, wounds of petioles. Fig. 32, attached leaf. Fig. 33, removed leaf. Fig. 34, attached leaf. Fig. 35, removed leaf. Fig. 36, attached leaf. Fig. 37, removed leaf.

meristem is so active that the wound will be completely filled with new tissue (fig. 36). This would seem to make the organ more solid than before activity started. Any dropping of tissue must have been due to the handling of the material and not from any natural abscission. More of the cicatrice tissue was developed in the leaf that was attached to the plant. If there is a water relation it might be shown here, since the leaf was allowed to dry at laboratory humidity.

The leaf that remained attached to the plant had more cells cut off between the meristem and the cells originally wounded. In this leaf there were twelve rows of cells between those of the original wound and the cell from which the meristem arose. In the detached leaf there were only eight cells at the most between the wound and the meristem. This would lead to the development of more pseudocicatrice in the leaf that remained on the plant.

## Summary

1. The development of the cicatrice and the possibility of abscission of that tissue were studied in the leaves of *Bryophyllum calycinum*.

2. Stages in the development of the pseudocicatrice and the cicatrice were traced from wounds made in the leaf at the margin, in the center, and at the petiole of the leaf.

3. Two series of leaves were used. One series was attached to the plant and the other was removed and allowed to dry under laboratory conditions. There was greater collapse and greater development of the pseudocicatrice in the leaf that was attached than in the leaf removed from the plant.

4. There was no evidence of abscission, either from the leaves that were attached to or from those removed from the plant.

5. The idea that abscission of tissues developed in response to wounds if the moisture supply to the leaf is high does not apply to *Bryophyllum calycinum*.

6. Any loss of tissue must have come from mechanical agitation rather than from true abscission.

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# MEGASPOROGENESIS AND DEVELOPMENT OF THE EMBRYO SAC OF CYPRIPEDIUM PARVIFLORUM

MARGERY C. CARLSON

## Literature review

A reinvestigation of megasporogenesis and the development of the embryo sac of *Cypripedium parviflorum* Salisb. seemed desirable because of the doubt expressed by some investigators concerning the so-called *Cypripedium*-type of embryo sac, which was based on PACE's (9) investigation of several species of this genus. SCHNARF (16) placed this type in his list of doubtful cases and called attention to the criticisms of PACE's work by RUTGERS (15). On the basis of RUTGERS' criticisms and of results of work on other species of *Cypripedium* by FRANCINI (5) and PROCINA (12), SCHNARF suggested that the embryo sac of *Cypripedium* develops according to the *Scilla*-type. MAHESHWARI (6, 7) agreed with SCHNARF and placed many members of the Orchidaceae in his *Allium*-type, which is the same as SCHNARF's *Scilla*-type.

PACE (9) concluded from her study of *C. parviflorum*, *C. spectabile* (*C. hirsutum* Mill.), *C. pubescens* (*C. parviflorum* var. *pubescens* [Willd.] Knight), and *C. candidum* that the archesporial cell, which functions as the megaspore mother cell, divides by meiosis and forms two daughter cells. The micropylar cell disintegrates and the chalazal one continues its development, resulting in the formation of the embryo sac. The nucleus of the chalazal daughter cell divides into two nuclei (megaspore nuclei), but the cell does not divide. An enlarging vacuole in the center pushes the two nuclei to opposite ends of the cell, where a second division takes place, producing a 4-nucleate embryo sac. One micropylar nucleus becomes the egg, the other a synergid; one chalazal nucleus migrates to the micropylar end of the embryo sac and becomes the other synergid, while the second chalazal nucleus becomes the polar nucleus. One synergid is then pushed upward by the entering pollen tube and unites with the polar nucleus. After discharge from the pollen tube, one male nucleus unites with the egg and the other unites with the two polar nuclei in the center of the sac. This was one of the first instances in which the account of embryo-sac development deviated from what is usually regarded as the normal.

RUTGERS pointed out the following inconsistencies in PACE's work. PACE stated that the mature embryo sac before fertilization has four nuclei but figured one with five. She showed no intermediate steps in the passage of one synergid to the center of the sac, where, she said, it unites with the polar nucleus. RUTGERS stated that PACE simply inferred this interpretation and called attention to one of her figures in which the remains of two synergids can be seen after double fertilization has occurred. RUTGERS gave his interpretation of PACE's figures as follows: The daughters of the primary chalazal nucleus[1] could not have become synergid and egg because of the polarization of the embryo sac; the primary chalazal nucleus must divide late and its two daughter nuclei remain close together and later both unite with one of the male nuclei; the primary micropylar

[1] Terminology used in this paper is that suggested by MAHESHWARI.

nucleus divides, one daughter becoming the egg and the other dividing and forming the two synergids; one synergid disintegrates when the pollen tube enters. RUTGERS states, "We do not claim to have given a decision, this being impos-

TABLE 1

| Species | Investigator and year |
|---|---|
| 1. Ordinarily normal (monosporic and 8-nucleate): | |
| Orchis pallens | Strasburger (19), 1878 |
| †Gymnadenia conopsea | Ward (22), 1880 |
| Habenaria ciliaris (Michx.) R. Br. | Brown (2), 1909 |
| H. integra (Nutt.) Spreng. | " " |
| Calopogon pulchellus R. Br. | Pace (10), 1909 |
| †*Epipactis pubescens (Willd.) A. A. Eaton | Brown and Sharp (3), 1911 |
| *Epipactis latifolia | Vermoesen (21), 1911 |
| *Epidendrum variegatum Hook. | Sharp (17), 1912 |
| E. verrucosum Sw. | " " |
| E. cochleatum L. | " " |
| E. globosum Jacq. | " " |
| Bletia shepherdii Hook. | " " |
| Coelogyne massangeana | " " |
| Pogonia macrophylla | " " |
| *Gyrostachis cernua | Pace (11), 1914 |
| G. gracilis | " " |
| *Orchis morio L. | Afzelius (1), 1916 |
| *Orchis sambucina L. | " " |
| Oncidium praetextum Rchb. fil. | " " |
| Coeloglossum viride (L.) Hn. | " " |
| Gymnadenia albida (L.) L. C. Rich | " " |
| Goodyera repens (L.) R. Br. | " " |
| Neottia nidus-avis | Modilewski (8), 1918 |
| Moringa oleifera Lam. | Puri (13), 1935 |
| Garcinia livingstonii T. Anders | Puri (14), 1939 |
| 2. Monosporic, normally producing less than 8-nuclei in embryo sac:‡ | |
| Garcinia kydia Roxb. | Treub (20), 1911 (doubted by Puri [14], 1939) |
| G. treubii Pierre | " " " |
| Phajus grandifolius Lour. | Sharp (17), 1912 |
| Corallorhiza maculata Raf. | " " |
| Broughtonia sanguinea R. Br. | " " |
| 3. Normally bisporic, thus belonging to Maheshwari's Allium-type: | |
| Cypripedium parviflorum | Pace (9), 1907 |
| C. spectabile (C. hirsutum Mill.) | " " |
| C. pubescens (C. parviflorum var. pubescens [Willd.] Knight) | " " |
| C. candidum | " " |
| †Paphiopedilum insigne (Wall.) Pfitz. | Afzelius (1), 1916 |
| †Cypripedilum guttatum Sw. | Procina (12), 1930 |
| Paphiopedilum leeanum | Francini (5), 1931 |
| †P. spicereanum (Rchb. fil.) Pfitz. | " " |
| †P. barbatum (Lindl.), Pfitz. | " " |
| †P. villosum (Lindl.), Pfitz. | " " |
| †P. venustum (Wall.), Pfitz. | " " |
| Achroanthes monophyllos (L.) Greene | Stenar (18), 1937 |

* A few of these embryo sacs reported bisporic; a few tetrasporic.

† Some mature embryo sacs may have less than 8 nuclei, either because the antipodal nuclei degenerate very early or because daughter nuclei of the primary chalazal nucleus do not divide.

‡ In these the chalazal nuclei do not divide after the 4-nucleate stage of the embryo sac, which is therefore 6-nucleate when mature.

sible without a reinvestigation of the whole material. But our suggestions must be admitted as a possible explanation and must be rejected on firm grounds before we can accept Miss PACE's."

RUTGERS described the development of the embryo sac in the orchid *Moringa oleifera* Lam. He found four megaspores in a T-arrangement, only one of which functions in forming the embryo sac. Two nuclear divisions occur normally, but the third division is restricted to one of the micropylar nuclei. The mature sac has, therefore, two chalazal nuclei which unite and become the secondary (fusion) nucleus, and three micropylar nuclei, two of which are synergids and one the egg. This sequence of events is similar to RUTGERS' suggestion of possible events in *Cypripedium*. However, PURI (**13**), reinvestigating *M. oleifera*, found the normal eight nuclei in the mature sac.

TREUB (**20**) described the course of development of the embryo sacs of *Garcinia kydia* Roxb. and *G. treubii* Pierre, and RUTGERS, probably influenced by TREUB, found the same course in *Moringa;* but PURI (**14**) found the normal eight nuclei in the mature sac of *G. livingstonii* T. Anders. Table 1 gives a classification of the work on the embryo sac of orchids.

## Material and method

The material used in this work was collected and prepared primarily for a study of seed formation, as described previously by CARLSON (**4**). Young flower buds were bagged, and when they had opened and their stigmas were receptive, they were pollinated and then rebagged. Ovaries were collected at intervals and were prepared for sectioning by the usual paraffin technique and were stained with safranin and fast green.

The relation of the stage of development of the ovules and their contents to the season and to the date of pollination was given in the earlier paper and will not be repeated here.

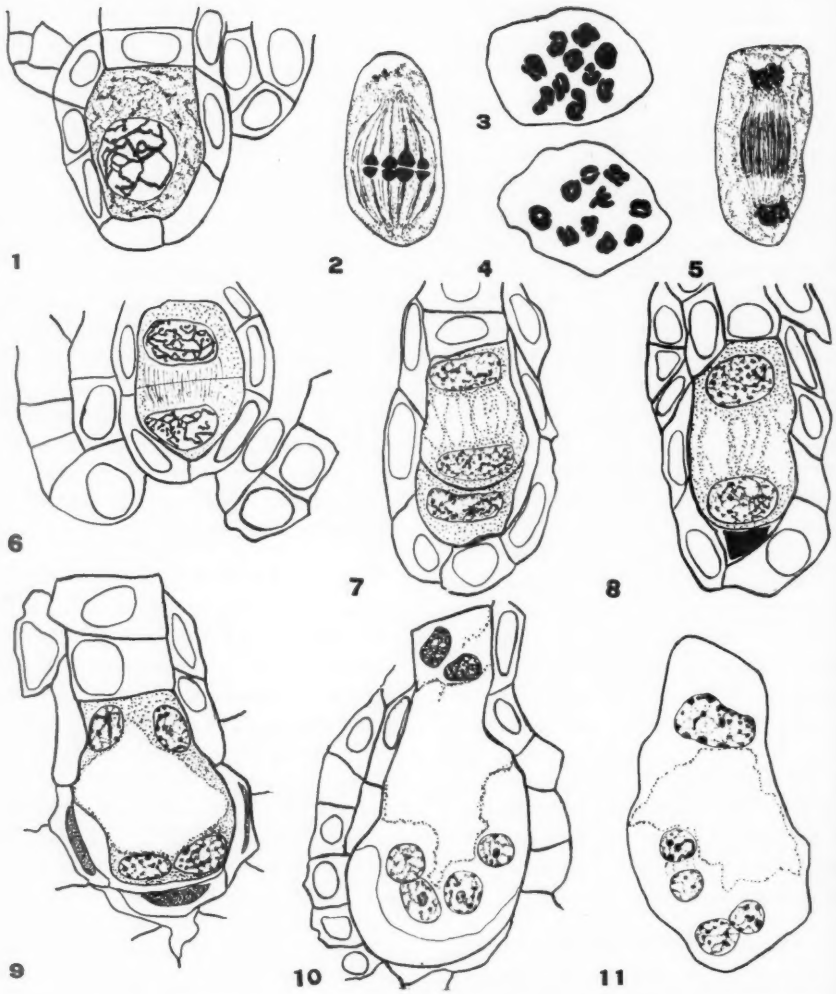
## Observations

### CHROMOSOMES

PACE (**9**) reported eleven pairs of chromosomes at the equator of the spindle in the metaphase of meiosis. I find ten pairs, as seen in figures 3 and 4. Many counts were made, and in no case where the chromosomes were sufficiently spread out to make the count certain were more than ten pairs found. The chromosomes are short and thick and vary somewhat in size and form. Even though they are small, it was possible to distinguish chromonemata and matrix.

### MEGASPOROGENESIS

The single hypodermal archesporial cell enlarges greatly and its nucleus undergoes the first meiotic division, thereby functioning directly as the megaspore mother cell (fig. 1). A prophase, metaphase, and telophase of this division are seen in figures 1, 2, and 5, respectively. When the two daughter nuclei are reconstructed, a wall is formed dividing the mother cell into two approximately equal-sized daughter cells—the dyads (fig. 6). The chalazal dyad begins to enlarge and the micropylar one soon shows signs of disintegration (fig. 7). The nucleus of the chalazal dyad now undergoes the second division, but the cell does not divide (figs. 7, 8). Many stages of this nuclear division were found, but no indication of even an ephemeral wall was seen. The two nuclei in the dyad, the result of two divisions following the megaspore mother nucleus, are equivalent to megaspore nuclei. Both take part in the formation of the embryo sac, making it bisporic.

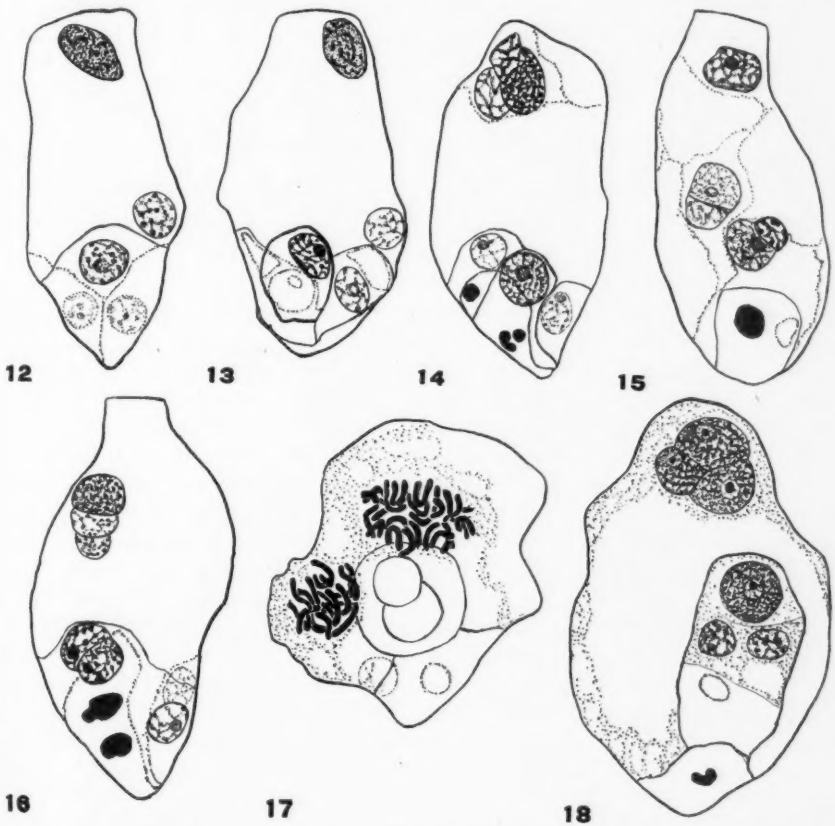



FIGS. 1–11.—Camera lucida drawings. All figures arranged so that chalazal end of ovule or embryo sac is toward top of page. Fig. 1, tip of ovule with lobe of inner integument showing; nucleus of enlarged megaspore mother cell in prophase of meiosis. Fig. 2, lateral view of heterotypic metaphase; only four of the ten pairs of chromosomes seen. Figs. 3, 4, polar views of heterotypic metaphase, showing ten pairs of chromosomes. Fig. 5, telophase of heterotypic division. Fig. 6, nuclear division completed; cell plate forming; each cell will be a dyad; lobe of inner integument extending around nucellus. Fig. 7, chalazal dyad elongated and its nucleus divided into two megaspore nuclei; micropylar dyad somewhat crushed. Fig. 8, chalazal dyad more elongated but no indication of cell division; vacuole developing between the two nuclei and initiating formation of bisporic embryo sac; micropylar dyad disintegrated. Fig. 9, each nucleus of embryo sac divided; micropylar dyad completely disappeared. Fig. 10, each nucleus at micropylar end of sac divided; those at chalazal end not divided and staining more heavily with safranin. Fig. 11, four nuclei at micropylar end of embryo sac; two nuclei at chalazal end have united.

### Embryo sac

A vacuole appears between the two nuclei in the chalazal dyad, which now becomes the embryo sac. As the vacuole enlarges, the embryo sac elongates and its nuclei are pushed to the ends of the sac (figs. 7, 8). Both nuclei divide, making four (fig. 9). Up to this point my observations agree with those of Pace, but beyond the 4-nucleate stage the events differ from those described by her.

The two nuclei at the micropylar end of the embryo sac divide and the four daughters lie free in the cytoplasm. The two chalazal nuclei, however, do not divide (fig. 10). This 6-nucleate stage was




Figs. 12–18.—Figs. 12, 13, egg and two synergids formed; polar nucleus free in inner cell; chalazal nuclei united. Fig. 14, end of pollen tube, with tube nucleus and two sperms, in embryo sac; two chalazal nuclei and polar nucleus lying in contact. Fig. 15, double fertilization; one sperm in contact with egg, other with polar nucleus; fused chalazal nuclei at inner end of sac. Fig. 16, double fertilization; one sperm in contact with egg; second sperm, polar nucleus, and fused chalazal nuclei in contact (latter group [4n] functions as primary endosperm nucleus). Fig. 17, polar view of embryo sac; 3-celled embryo; division of two groups of primary endosperm nuclei. Fig. 18, young embryo with 3-celled embryo proper and 1-celled suspensor; fused chalazal nuclei, polar nucleus and sperm in contact; pollen tube with tube nucleus still visible but synergids have disappeared.

missed by PACE, but it appears frequently in the present material.

Three of the micropylar nuclei now form the typical egg apparatus, and the fourth (upper polar) moves to the center of the sac (figs. 12, 13). The two chalazal nuclei may remain apart, they may approach and lie in contact, or they may fuse (figs. 12, 13). Sometimes they unite before the egg apparatus is constructed (fig. 11), and sometimes they are still apart after the embryo has started to form. The upper polar nucleus may fuse with the two chalazal nuclei but usually simply lies in contact with them (fig. 14).

### FERTILIZATION

The pollen tubes grow downward on either side of the base of each of the three branching placentae. Great masses of them follow along the surface of the placentae. They reach the ovules when meiosis is in progress or during the early stages of embryo-sac development. The tube nucleus and the two male nuclei follow in succession down the tube and may easily be found just back of its tip. When a pollen tube enters the embryo sac (fig. 14) it sometimes becomes difficult to find the synergids (fig. 15), but often both may be found as late as fertilization (fig. 16). The tube and male nuclei change their shapes and staining reactions when the tube enters the sac (fig. 14). After discharge from the ube, the male nuclei enlarge and migrate into the sac, where one unites with the egg (figs. 15, 16).

The history of the second sperm depends on what has happened in the lower end of the sac. As already described, the two chalazal and the upper polar nuclei may not actually fuse but merely lie in contact, or the two chalazal nuclei may fuse and this double nucleus may lie in contact with the upper polar one. In either case the second sperm usually moves into contact with, but does not fuse with, the group (figs. 16, 18). Sometimes the polar nucleus does not join the chalazal nuclei but unites with the sperm independently (figs. 15, 17). There is no triple-fusion nucleus in the ordinary sense of the term. Because this group of nuclei is concerned with the formation of endosperm, it may be called the primary endosperm nucleus or group of nuclei. Frequently, one or two dark-staining bodies remain in the pollen tube after fertilization (fig. 16).

### EMBRYO AND ENDOSPERM

The early stages of embryo development have already been described by CARLSON and by PACE, but 3- and 4-celled stages may be seen in figures 17 and 18. The primary endosperm nucleus, or group of nuclei, contains a tetraploid chromosome complement. PACE shows a metaphase and telophase in the first division of the primary endosperm nucleus and also illustrates embryo sacs with two and four large endosperm nuclei. In most of the sacs in my material the nuclei, which should form the primary endosperm nucleus, neither fuse nor divide, but figure 17 shows an end view of an embryo sac with metaphases or early anaphases of two nuclei which started to divide before uniting. The embryo in this figure is in the 3-celled stage. Whether or not the primary endosperm nucleus divides, it or its descendants soon disintegrate and the mature seed has no endosperm.

## Discussion

This study confirms PACE's conclusion that two megaspore nuclei enter into the formation of the embryo sac, but corrects

her ideas about the fate of the nuclei after the 4-nucleate stage of the sac. The micropylar nucleus of the dyad functions normally, but the one at the opposite end divides although its daughters do not. They are variable in their behavior, usually fusing and together functioning as the upper polar nucleus. There are no antipodal cells as ordinarily defined. Fertilization of the egg is normal, but the other "fertilization" is variable. The primary endosperm nucleus, or group of nuclei, which is 4n, may or may not divide, forming endosperm nuclei. RUTGERS' guess that the daughters of the primary chalazal nucleus fuse and then unite with a male nucleus was right, but he was mistaken in thinking that no polar nucleus was formed in the micropylar end of the sac. Variability in the chalazal end of the sac is not unusual. It occurs in those plants marked with † in table 1.

Most of the orchids which have been investigated have normal, monosporic, 8-nucleate embryo sacs, but some are monosporic with 6-nucleate sacs. *Cypripedium parviflorum*, *C. hirsutum*, *C. pubescens*, *C. candidum* (9), *C. guttatum* (12), *Achroanthus monophyllas* (18), and six species of *Paphiopedilum* (1, 5), are bisporic and 6-nucleate. The so-called *Cypripedium*-type of embryo-sac development must be abandoned and the bisporic types placed in the *Scilla*-type of SCHNARF, or the *Allium*-type of MAHESHWARI, who claims that the name "*Allium*-type" has priority.

## Summary

1. The haploid number of chromosomes in *Cypripedium parviflorum* is ten rather than eleven, as previously reported.

2. Two potential megaspore nuclei enter into the formation of the embryo sac, making it bisporic, and placing this species of *Cypripedium* in the *Scilla*-type of SCHNARF, or the *Allium*-type of MAHESHWARI.

3. After the 4-nucleate stage of the embryo sac, the two micropylar nuclei divide and two of the resulting four nuclei become synergids, one becoming the egg and the other the polar nucleus. The two chalazal nuclei do not divide and usually fuse. They may, however, simply lie in contact. At this stage the embryo sac contains either six or five nuclei, depending on whether the chalazal nuclei have fused.

4. The polar nucleus from the micropylar group usually migrates into contact with the fused or contiguous chalazal nuclei and may never actually fuse with them.

5. Double fertilization occurs. One sperm fuses with the egg and the second may unite with or lie in close contact with the chalazal group of nuclei.

6. The primary endosperm group of nuclei, containing the tetraploid number of chromosomes, may not divide or it may reach a few-nucleate stage before disintegrating.

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# EFFECT OF COMMERCIAL FERTILIZERS ON THE SEX EXPRESSION OF HEMP[1]

C. A. BLACK

## Introduction

Owing to the shortage of hard fibers in the United States during the early part of the war, hemp was grown in several states for military and domestic needs. Except for Kentucky and Wisconsin, hemp had not been grown previously to any extent in the states selected for production, and relatively little was known about the successful culture of the crop in the new areas. For this reason, a number of field experiments with commercial fertilizers were undertaken to determine their value in hemp production. These experiments, while designed primarily to obtain data on yield, furnished a good opportunity to study the effect of fertilizers on the sex ratio of hemp under field conditions. Practical interest in the problem arises from the difference in the nature and quantity of fiber obtained from plants of the different sexes. The male plants contain a harsher fiber and a higher percentage of it than do the female plants (3). The present paper records the results of sex counts obtained from experiments conducted in Iowa in 1943 and 1944.

## Review of literature

Several investigators have found it possible to bring about a change in the sex expression of certain plants by varying the environmental conditions. In the case of hemp, the factors of light, tem-

[1] Journal paper no. J 1363 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project no. 825.

perature, and nutrient supply have been shown to be of importance. By varying the photoperiod, SCHAFFNER (8) was able to control the degree of sex reversal. Where the length of the photoperiod was short, more than 90% of the plants showed sex reversal, the male to the female and vice versa. NELSON (6) noted that in both wild and fiber hemp, low temperature (15° C.) favored a dominance of female plants. The effect was more pronounced when the aerial portion of the plant was subjected to the low temperature than when the roots were kept at the low temperature.

No particular effect of nutrition was noted by earlier investigators. The data reviewed by HEYER (5) were negative. It was concluded, therefore, that sex was determined in the seed and not influenced by external conditions.

BOSE (2), working with hemp, reported some counts made in a portion of a field which had been fertilized with mustard cake (an organic fertilizer containing nitrogen, together with some phosphorus and potassium). The relative numbers of males and females were almost identical on the treated and untreated areas. Additional counts were made on a field of four plots, three receiving commercial fertilizers and one serving as a check. The ratio of males to females varied from 1:0.92 on the check plot to 1:1.27 on the ammonium-sulphate plot. Superphosphate and Ammophos gave intermediate values. Although the differences probably are within the limits of sampling variation, the hemp on all the fertilized areas had a smaller proportion of males than had the check; furthermore, both the plots receiving nitrogen had a lower percentage of males than had the two plots not fertilized with nitrogen.

Later work by TIBEAU (10), conducted in the greenhouse, showed that by varying the nitrogen supply it was possible to produce all male, all female, or a population of male and female plants. When no nitrogen was supplied, all the plants were male. When the plants were grown with a "normal" supply (that furnished in Knop's solution), 65% of the plants were female and 35% were male. With eight times the "normal" concentration of nitrogen, all the plants were female.

The results of TIBEAU (10), in conjunction with those of TALLEY (9), GARDNER (4), and others, suggest that the carbohydrate-nitrogen balance in certain plants influences the sex expression. It seems that, in general, high-nitrogen low-carbohydrate plants tend toward femaleness, whereas low-nitrogen high-carbohydrate plants tend toward maleness.

### Experimental methods

In 1943, the relative numbers of male and female plants were counted in two experiments. The treatments employed were commercial ammonium sulphate, superphosphate, and potassium chloride added at rates to furnish, respectively, N at 25 pounds per acre, $P_2O_5$ at 50 pounds per acre, and $K_2O$ at 25 pounds per acre. Each treatment was used singly and in all combinations with each of the others in a factorial design having four replications. In 1944, counts were made on six experiments. The 1944 experiments included N at 50 and 100 pounds per acre, $P_2O_5$ at 30 pounds per acre, and $K_2O$ at 20 pounds per acre, again in a factorial design with four replications (three replications in experiments 12 and 13).

The fertilizers were applied on small plots, 10 × 13 feet in 1943 and 13 × 16 feet in 1944. The sample of hemp taken from each plot consisted of a composite

of six 24 × 26 inches of sub-samples in 1943 and of five 36 × 36 inches of sub-samples in 1944. In making the sex counts, small lots of about ten plants each were taken at random from the composite sample. The male and female plants were counted before another lot was taken. This process was repeated until a total of 100 male plus female plants had been counted per plot. No hermaphroditic specimens were noted, although there may have been a few which escaped attention. In each experiment it was found necessary to discard a few plants which were not far enough advanced to be recognized definitely as either male or female. These were small plants which presumably germinated late and were stunted by lack of light under the foliage of the earlier plants.

The results of the counts are expressed in this report in terms of the percentage of male plants, it being understood that the percentage of female plants represents the difference between that and 100.

### Results and discussion

In both 1943 and 1944 it was found that fertilizers affected the yield of hemp. In general, nitrogen was the major limiting factor, followed in order by phosphorus and potassium. Table 1 summarizes the effects of fertilizers on the yield of hemp for those experiments on which counts of male and female plants were made.

TABLE 1

EFFECT OF NITROGEN, PHOSPHORUS, AND POTASSIUM FERTILIZERS ON YIELD OF GREEN HEMP

| Field no.* | Yield without fertilizer (tons per acre) | Average yield increase with treatment† (tons per acre) | | | |
|---|---|---|---|---|---|
| | | N | | P | K |
| 3 | 8.21 | 1.99‡ | | 0.79 | −0.94 |
| 6 | 7.02 | 2.17‡ | | 0.37 | −0.59§ |
| | | $N_1$ | $N_2$ | P | K |
| 10 | 10.16 | 2.81‡ | 4.06‡ | −0.26 | 0.54§ |
| 11 | 10.54 | 2.90‡ | 3.57‡ | −0.46 | 0.64 |
| 12 | 8.73 | 2.21‡ | 2.98‡ | 0.58§ | 0.26 |
| 13 | 11.23 | 1.11‡ | 1.63‡ | 0.37 | 0.20 |
| 14 | 14.32 | 0.91 | 1.38‡ | 1.07‡ | −0.47 |
| 15 | 3.25 | 0.52 | 1.23‡ | −0.09 | −0.24 |

* For further details regarding yields, soil types, past management, location, etc., see BLACK and VESSEL (1).

† Acre applications of fertilizer ingredients as follows: For fields 3 and 6: N 25 lb. N; P 50 lb. $P_2O_5$; K 25 lb. $K_2O$. For fields 10–15: $N_1$ 50 lb. N; $N_2$ 100 lb. N; P 30 lb. $P_2O_5$; K 20 lb. $K_2O$.

‡ Significant at 1% level.

§ Significant at 5% level.

The results of counts made on the 1943 experiments are shown in table 2. It will be noted that about half the plants were male, regardless of treatment. The only difference which reached significance at the 5% level was that for phosphorus on field 6, where hemp on phosphorus-treated plots averaged 52% male and that without phosphorus averaged 48%. In the final yields on field 6 there was no

significant response to phosphorus. Early in the season, however, definite response to phosphorus was evident. The reverse trend in percentage of male plants was found on field 3, where there was more marked early response to phosphorus and where phosphorus produced twice as much increase in the final yields.

The hemp on both field 3 and field 6 was rather low-yielding, owing in the main to a deficiency of nitrogen. Even the nitrogen-fertilized hemp was deficient throughout a considerable part of the growing season. To obtain some indication as to the effect of a greater supply of nitrogen, counts were made on a heavily fertilized, non-replicated strip at the side of experiment 3. The fertilizers applied to this area included phosphorus and potassium at the same rates as those used in the experiment, and nitrogen at the rate of 100 pounds per acre—four times the rate in the experiment. Hemp on this more heavily fertilized area did not contain discernible quantities of nitrate nitrogen after midseason, but should have had an adequate supply earlier in the season.[2] Sex counts showed the presence of 49% male plants, a value well within the range obtained on the adjacent experiment.

One replicated experiment was conducted in 1943 in which nitrogen was applied at the rate of 100 pounds per acre to a field which gave a high yield of hemp without fertilization[3] and on which the nitrogen-fertilized hemp gave a test for nitrate nitrogen throughout the season. Counts made on composite samples from all replications gave 50% male on the hemp without additional nitrogen and 49% on the hemp which received the heavy application of nitrogen.

TABLE 2

EFFECT OF NITROGEN, PHOSPHORUS, AND POTASSIUM FERTILIZERS ON PERCENTAGE OF MALE HEMP PLANTS; 1943 EXPERIMENTS

| Field no. | Percentage males with treatments* | | | | | | | | Average effects | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | None 0-0-0 | N 5-0-0 | P 0-10-0 | NP 5-10-0 | K 0-0-5 | NK 5-0-5 | PK 0-10-5 | NPK 5-10-5 | N | $N_1$ | $P_0$ | $P_1$ | $K_0$ | $K_1$ |
| 3 | 56.0 | 52.0 | 52.0 | 47.0 | 49.0 | 48.0 | 47.0 | 48.0 | 51.0 | 48.7 | 51.2 | 48.5 | 51.7 | 48.0 |
| 6 | 47.7 | 49.2 | 50.0 | 52.7 | 46.0 | 49.0 | 52.2 | 53.0 | 49.0 | 51.0 | 48.0 | 52.0† | 49.9 | 50.1 |
| Average | 51.9 | 50.6 | 51.0 | 49.9 | 47.5 | 48.5 | 49.6 | 50.5 | 50.0 | 49.9 | 49.6 | 50.2 | 50.8 | 49.0 |

* 500 pounds of each fertilizer per acre.
† Significant difference at 5% level.

The results of counts made on the 1944 experiments are shown in table 3. Since the 25-pound rate of nitrogen used in 1943 was clearly inadequate in many cases, nitrogen was applied at 50 and 100 pounds per acre in 1944, with the assumption that 100 pounds would be adequate on the poorer fields and excessive on the better fields. As a result of heavy rains in the spring and early summer, fields 12, 13, and 15 were not planted for some time after the fertilizers had been

[2] Acre yield of green hemp was 14.87 tons.

[3] Acre yield of green hemp was 16.85 tons on the plots without nitrogen and 21.20 tons on the plots which received nitrogen at the rate of 100 pounds per acre.

TABLE 3

EFFECT OF NITROGEN, PHOSPHORUS, AND POTASSIUM FERTILIZERS ON PERCENTAGE OF MALE HEMP PLANTS; 1944 EXPERIMENTS

| FIELD NO. | PERCENTAGE MALES WITH TREATMENT (500 POUNDS FERTILIZER PER ACRE) | | | | | | | | | | | | Average effects | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | None 0-0-0 | $N_1$ 10-0-0 | $N_2$ 20-0-0 | P 0-6-0 | $N_1P$ 10-6-0 | $N_2P$ 20-6-0 | K 0-0-4 | $N_1K$ 10-0-4 | $N_2K$ 20-0-4 | PK 0-6-4 | $N_1PK$ 10-6-4 | $N_2PK$ 20-6-4 | $N_0$ | $N_1$ | $N_2$ | $P_0$ | $P_1$ | $K_0$ | $K_1$ |
| 10 | 50.0 | 49.0 | 53.5 | 50.2 | 47.5 | 42.5 | 49.0 | 48.2 | 50.5 | 49.5 | 50.2 | 44.7 | 49.7 | 48.7 | 47.8 | 50.0 | 47.5† | 48.8 | 48.7 |
| 11 | 55.0 | 46.0 | 57.7 | 55.5 | 55.0 | 50.7 | 58.7 | 49.2 | 48.7 | 50.0 | 48.5 | 48.5 | 54.8 | 49.7 | 51.4 | 52.6 | 51.4 | 53.3 | 50.6 |
| 12 | 48.0 | 52.0 | 49.3 | 50.0 | 49.0 | 43.0 | 51.0 | 44.0 | 43.7 | 46.7 | 47.7 | 50.3 | 48.9 | 48.2 | 46.6 | 48.0 | 47.8 | 48.6 | 47.2 |
| 13 | 51.0 | 47.7 | 50.3 | 50.3 | 46.3 | 50.3 | 51.0 | 49.0 | 49.0 | 51.3 | 49.3 | 42.7 | 50.9 | 48.1 | 48.1 | 49.7 | 48.4 | 49.3 | 48.7 |
| 14 | 45.0 | 44.2 | 46.2 | 41.2 | 44.0 | 44.7 | 43.5 | 48.7 | 48.0 | 51.0 | 48.5 | 50.7 | 45.2 | 46.4 | 47.4 | 46.0 | 46.7 | 44.2 | 48.4* |
| 15 | 46.7 | 44.7 | 45.0 | 44.0 | 49.5 | 47.2 | 46.2 | 51.7 | 45.0 | 41.2 | 46.2 | 47.0 | 44.6 | 48.1 | 46.1 | 46.6 | 45.9 | 46.2 | 46.2 |
| Average‡ | 50.2 | 46.7 | 52.1 | 49.3 | 48.3 | 46.9 | 50.5 | 48.8 | 49.1 | 50.4 | 49.1 | 46.9 | 50.1 | 48.2 | 48.7 | 49.6 | 48.5 | 48.9 | 49.1 |
| Average§ | 49.3 | 47.0 | 50.4 | 48.4 | 48.6 | 46.4 | 49.8 | 48.7 | 47.6 | 48.2 | 48.4 | 47.4 | 48.9 | 48.2 | 48.0 | 48.8 | 47.9 | 48.4 | 48.4 |

* Difference significant at 1% level.

† Difference significant at 5% level.

‡ Omits fields 12 and 15, on which the nitrogen-fertilized hemp was deficient in nitrogen. § Includes all fields. Experiments 12 and 13 each had three replications, whereas all others had four. Each replication has been given equal weight in the averages, which gives experiments 12 and 13 somewhat less weight than the others.

applied. Apparently there was opportunity for loss of some of the nitrogen by leaching before the hemp was planted, because even the hemp on plots receiving 100 pounds of nitrogen per acre was deficient in nitrogen on fields 12 and 15. Because of this difference in the nitrogen status of the different experiments, the data have been averaged in two groups, one including only those experiments (10, 11, 13, and 14) where the nitrogen-fertilized hemp had an adequate supply of nitrogen and the other including all experiments.

Again in 1944 all treatments averaged about 50% male (the average of all experiments was 48.4%). There was a slight tendency for the percentage of males to decrease as both nitrogen and phosphorus were increased. The lowest percentages occurred on the plots which received both nitrogen (at the 100-pound rate) and phosphorus. The analysis of the combined data from all six experiments indicated a significant interaction of nitrogen and phosphorus.

Statistically significant differences occurred in two individual experiments. On field 10, the phosphorus-treated plots had significantly fewer males. The depression in number of males occurred entirely at the highest level of nitrogen ($N_2$) and resulted in a highly significant N-P interaction. On field 14 there was a highly significant increase in the percentage of male plants on the plots which received potassium. The increase from potassium was four times as great in the presence of phosphorus as in the absence of phosphorus, a significant interaction.

Field 10 gave an increase in acre yield of 4.06 tons from nitrogen and 0.54 tons from potassium, and a decrease of 0.26 tons from phosphorus. The acre yield of hemp on field 14 was increased 1.38 tons from nitrogen and 1.07 tons from phosphorus, and was decreased 0.47 tons from potassium. Thus, nitrogen, which had the greatest effect on the yield of hemp and might be expected to have the greatest effect on the relative numbers of male and female plants, was the only fertilizer constituent which produced no significant main effects. The fertilizers which significantly affected the relative numbers of male and female plants (phosphorus in experiment 10 and potassium in experiment 14) were ingredients which had little or no effect on the yield. The same may be said for the 1943 results.

No physiological explanation for the results obtained is apparent, and yet it would be unusual to obtain three statistically significant main effects in eight experiments purely as a matter of chance. The changes were small, however, and the effects were of no practical importance in producing hemp consisting largely of either male or female plants. The conditions necessary to produce hemp of this nature evidently lie outside the range produced in these experiments.

As regards the use of nitrogen for producing a stand of female plants, it would appear that the quantity required under field conditions would be extremely high. Moreover, present indications are that weak fiber would be obtained from such high-nitrogen plants. On the other hand, to produce a preponderance of male plants by withholding nitrogen apparently would require such marked nitrogen deficiency that only a very low yield of fiber could be obtained.

## Summary

Eight field experiments with hemp were conducted in Iowa in which was determined the effect of nitrogen, phosphorus, and potassium fertilizers on the relative numbers of male and female plants. Phosphorus produced a statisti-

cally significant increase in percentage of male plants in one experiment and a decrease in another. Potassium produced a significant increase in percentage of male plants in one experiment. No significant effects were produced by nitrogen. All differences due to fertilizers were small, the sex ratios varying but little between treatments.

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# EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE READILY AVAILABLE CARBOHYDRATE CONSTITUENTS IN ANNUAL MORNING-GLORY

JOHN W. MITCHELL[1] AND JAMES W. BROWN[2]

## Introduction

The use of certain growth-regulating substances as herbicides has recently been considered (**1, 2, 3, 4, 7, 8, 9**). It has been reported that the physiological changes associated with the death of plants treated with toxic amounts of 2,4-dichlorophenoxyacetic acid and some other related compounds occur at a relatively slow rate, so that the plants may live for 2–4 weeks after treatment (**3, 4, 7**).

In earlier experiments, naphthaleneacetic acid and some other compounds accelerated the rate of starch hydrolysis and depleted the supply of readily available carbohydrate reserves in bean leaves when these substances were applied as emulsion sprays (**5, 6**).

The present experiments were undertaken to determine the effect of spray application of toxic amounts of 2,4-dichlorophenoxyacetic acid on the readily available carbohydrate reserves of an-

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nual morning-glory plants, *Ipomoea lacunosa* L., some of which were grown under conditions of photoperiod and soil fertility favoring vigorous vegetative growth and others under less favorable conditions.

## Experimentation

### Preliminary test

In a preliminary experiment, morning-glory plants were grown in composted soil contained in 4-inch clay pots under greenhouse conditions, during the months of January, February, and March, 1945. During this period the plants grew only a limited amount vegetatively but produced numerous flowers and seeds. Approximately 2 months after planting, the plants were 4–6 inches tall, bushy, and had matured four to ten seedpods per plant. One hundred uniform plants were selected, and two-thirds of them were sprayed with an aqueous mixture containing 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500. The foliage of each plant was thoroughly covered with this solution. The remaining plants were left untreated as controls. Samples consisting of three treated and three untreated plants of average size were collected at intervals of 2–4 days.

Microchemical estimations of the amount of starch in the plants were made by cutting transverse sections of stem tissue at one-fourth and at one-half the distance from the apex to the ground level, at the ground level, and also a section was cut through the upper part of the root. These sections were placed in iodine solution and the amount of coloration due to the starch content in the tissue was noted.

Two weeks after treatment, microchemical tests indicated that the starch reserve had greatly decreased in the stems of treated plants. At the end of 3 weeks only a trace remained in these stems, while those of untreated plants contained an abundance of starch. At this time the leaves of the treated plants were brown and curled, the stems were beginning to dry, and the plants were near death. The untreated plants were green and healthy in appearance, although they were not growing vegetatively.

### Nonvegetative plants

Methods.—Morning-glory plants were grown from seed under greenhouse conditions in lightly composted soil contained in 4-inch clay pots. Small pots and soil of low fertility were used, in order to provide conditions unfavorable for vigorous vegetative growth. On March 20 the plants were 3–6 inches tall, and 330 were selected for size and uniformity. One-half of these were sprayed with an aqueous mixture containing 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500. The foliage was thoroughly covered with the spray mixture. In order to inhibit excessive vegetative growth and hasten fruit production, both treated and untreated plants were covered with a black cloth so as to permit but 10 hours of natural illumination.

Thirty treated and thirty untreated plants were harvested at intervals during the next 20 days. Plants in these samples were divided into roots, hypocotyls, stems, flower buds, and leaves, and all like parts were combined to make five individual samples for each treatment. The samples were dried at 80° F., ground, and analyzed for total reducing sugars and for dextrin and starch by means of saliva digestion (6).

RESULTS.—Within 24–48 hours slight epinasty and stem curvatures developed on treated plants. Treatment inhibited vegetative growth (table 1), and the leaves of the sprayed plants became yellow within 2 weeks after treatment. New flower buds failed to develop on the treated plants, and those that were partially developed at the time of treatment became discolored and died within 6–10 days. The flower buds were the first part to show necrosis as a result of treatment and the first to die.

TABLE 1

FRESH WEIGHT OF UNTREATED MORNING-GLORY PLANTS COMPARED WITH THAT OF PLANTS SPRAYED WITH AQUEOUS MIXTURE CONTAINING 1000 P.P.M. 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. FIGURES EXPRESSED IN GRAMS AND OBTAINED FROM SAMPLES OF THIRTY TREATED AND THIRTY UNTREATED GREENHOUSE PLANTS

| PLANT PARTS | DAYS AFTER TREATMENT | | | | |
|---|---|---|---|---|---|
| | 0 | 8 | | 20 | |
| | | Control | Treated | Control | Treated |
| Roots | 31.5 | 24.6 | 33.3 | 38.0 | 12.0 |
| Hypocotyls | 8.0 | 8.6 | 9.0 | 8.3 | 5.6 |
| Stems | 24.1 | 23.6 | 25.4 | 29.0 | 14.4 |
| Buds | 6.7 | 16.1 | 7.2 | 35.0 | 1.7 |
| Leaves | 71.0 | 57.0 | 60.0 | 40.0 | 16.1 |
| Total | 141.3 | 129.9 | 134.9 | 150.3 | 49.8 |

Thirty untreated plants developed 130 mature fruits and forty-four immature ones during the experiment, while treated plants failed to develop fruit. Twenty days after treatment, the foliage and stems of the sprayed plants were discolored, on many of the plants a region of stem near the soil surface was partially decomposed as a result of the growth of fungi and bacteria in the stem tissues, and the plants were designated as dead (fig. 1). Ninety-five per cent of the roots of the sprayed plants were classified as dead 20 days after treatment, and the remaining ones appeared beyond recovery.

Untreated plants failed to increase appreciably in size during the experiment, since they were subjected to environmental conditions which did not favor vigorous growth (table 1). The average dry weight of the treated plants decreased by approximately 60%, which may have been due in part to digestion by microörganisms during the latter part of the experiment.

The sugar content of sprayed plants increased markedly up to about the eighth day, when they contained 73% more sugar than did the controls; then the sugar content of the sprayed plants decreased, until at the end of the experiment they contained only about one-third as much sugar as did the unsprayed ones (table 2). The most marked increase in sugar content as the result of treatment occurred in the leaves. In contrast, the sugar content of the flower buds of the treated plants remained consistently below that of buds of the untreated ones. In treated plants no sugar could be detected in the tissues of the buds, and there was only a trace of it in the roots on the twentieth day following treatment.

The starch-dextrin content of the sprayed plants decreased markedly, and on the twentieth day following treatment they contained only about one-fifteenth as much starch and dextrin as did the unsprayed plants. The most rapid rate of hydrolysis of starch occurred in the leaves, buds, and stems in the treated plants (table 2).

The percentage of readily available carbohydrates (sugar, starch, and dextrin) decreased in the untreated and

FIG. 1.—Morning-glory plants subjected to 10-hour photoperiod and grown in relatively infertile soil to inhibit vegetative growth. Untreated (left) compared with plants sprayed with aqueous mixture containing 1000 p.p.m. 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500 (right). Twenty-one days after treatment.

TABLE 2

TOTAL SUGAR AND STARCH-DEXTRIN CONTENTS OF MORNING-GLORY PLANTS GROWN IN GREENHOUSE UNDER CONDITIONS OF PHOTOPERIOD AND SOIL FERTILITY UNFAVORABLE FOR VIGOROUS VEGETATIVE GROWTH AND SPRAYED WITH AQUEOUS MIXTURE CONTAINING 1000 P.P.M. 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. FIGURES REPRESENT MILLIGRAMS OF CARBOHYDRATES IN THIRTY PLANTS

| Days | Roots | | Hypocotyls | | Stems | | Buds | | Leaves | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C* | T | C | T | C | T | C | T | C | T | C | T |
| | Sugar | | | | | | | | | | | |
| 0 | 288 | ..... | 36 | ..... | 139 | ..... | 36 | ..... | 190 | ..... | 689 | ..... |
| 3 | 216 | 228 | 49 | 87 | 144 | 230 | 46 | 33 | 260 | 660 | 715 | 1238 |
| 8 | 232 | 200 | 56 | 183 | 138 | 322 | 197 | 51 | 133 | 281 | 756 | 1037 |
| 15 | 136 | 38 | 55 | 152 | 118 | 191 | 262 | 10 | 46 | 43 | 617 | 434 |
| 20 | 170 | 6 | 30 | 98 | 86 | 100 | 349 | 0 | 128 | 17 | 763 | 221 |
| | Starch-dextrin | | | | | | | | | | | |
| 0 | 778 | ..... | 713 | ..... | 925 | ..... | 28 | ..... | 1304 | ..... | 3748 | ..... |
| 3 | 633 | 438 | 505 | 544 | 1333 | 682 | 63 | 22 | 1006 | 612 | 3540 | 2298 |
| 8 | 574 | 258 | 836 | 416 | 1341 | 480 | 252 | 51 | 725 | 15 | 3728 | 1220 |
| 15 | 633 | 94 | 484 | 107 | 702 | 81 | 851 | 2 | 58 | 0 | 2728 | 284 |
| 20 | 611 | 18 | 321 | 17 | 404 | 0 | 675 | 0 | 270 | 0 | 2281 | 35 |

* C, control; T, treated.

treated plants during the experiment. A decrease in the amount of these constituents in the untreated plants was to be expected, since both sets of plants were subjected to adverse conditions of photoperiod and soil fertility.

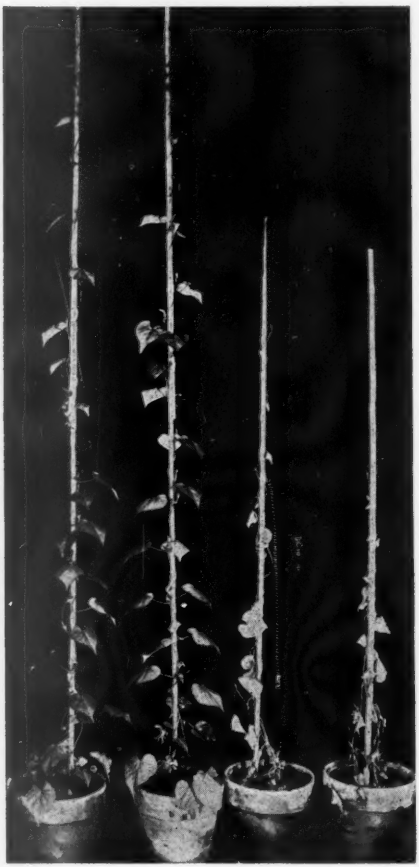

FIG. 2.—Morning-glory plants exposed to 12- to 13-hour photoperiod and grown in relatively fertile soil to hasten vegetative growth and seed production. Two untreated plants (left) compared with two plants sprayed with aqueous mixture containing 1000 p.p.m. 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500 (right). Twenty-one days after treatment.

### VEGETATIVE PLANTS

METHODS.—Morning-glory plants were grown under greenhouse conditions in heavily composted soil contained in 6-inch clay pots. On April 8, approximately 600 plants were selected for size and uniformity and arranged on two greenhouse benches. Alternating rows of these plants were then sprayed with an aqueous mixture containing 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500. The remaining plants were left untreated. In order to maintain vigorous vegetative growth, the soil in which the plants were growing was treated with a solution containing 2000 p.p.m. of calcium nitrate during the first week following treatment and the surface of the soil in each pot was covered with well-rotted manure.

Thirty treated and thirty untreated plants were harvested at intervals during the 20-day period immediately following treatment. They were divided into roots, hypocotyls, stems, flower buds, and leaves. These samples were then dried at 80° F., ground, and analyzed for total reducing sugars and for starch and dextrin (6).

RESULTS.—The sprayed plants showed marked epinasty within 24 hours after treatment, but the leaves remained green until approximately the sixth day, when most of those on the treated plants changed from green to red and yellow and later to a brownish color. The sprayed plants failed to grow in length following treatment, while an average increase of approximately three expanded internodes was recorded for the untreated plants. The total average dry weight of the sprayed plants decreased slightly during the 3-week period following treatment, while that of the untreated plants increased by 175% during

the same period. Plants sprayed with the mixture containing 1000 p.p.m. of the acid did not exhibit swelling of tissues or external evidence of gall formation, as has been reported in connection with the use of equal concentrations of this acid on other species of plants (4).

The development of buds on treated plants was noticeably inhibited during plants decreased from 18.4% to 2.1% (dry-weight basis), while that of controls increased slightly during the 3-week period immediately following treatment (fig. 3). Sugar at first accumulated in the treated plants, and on the sixth day they contained approximately twice as much as did the untreated ones. After 3 weeks, however, the treated plants contained

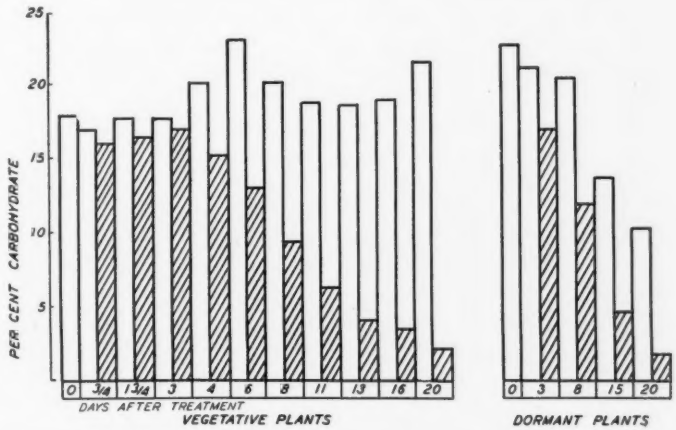


FIG. 3.—Percentage of carbohydrates (sugar, starch, and dextrin) in vegetatively vigorous and in relatively dormant morning-glory plants treated with aqueous mixture containing 1000 p.p.m. 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500 (shaded bars), compared with that in untreated plants (unshaded). Values represent carbohydrate contained in samples from thirty plants collected at successive intervals following treatment.

the first 3 days following treatment. On the fourth day untreated plants had an average of three open flowers per plant, while no flowers had opened on the sprayed ones on this date. Two weeks after treatment, 35% of the roots and all the leaves of the treated plants were dead. Decayed areas had developed at the ground level on stems of nearly all the treated plants. Three weeks after treatment the stems, buds, leaves, and 95% of the roots of the sprayed plants were dead (fig. 2).

The readily available carbohydrates (sugars, starch, and dextrin) in sprayed only 15.9% as much sugar as did the unsprayed.

The greatest increase in sugar content as the result of treatment occurred in the leaves. They contained three to eight times as much sugar as did the leaves of unsprayed plants during the 4–8 days following treatment. In contrast, the sugar content of the flower buds of treated plants did not exceed that of buds on the unsprayed plants, but instead the amount in the treated ones decreased rapidly during the 2 weeks immediately following treatment, and at the end of this period they contained only a trace

of sugar (table 3). Only 18 hours after treatment the flower buds of the treated plants contained approximately 46% less sugar than did the buds of untreated plants.

flower buds of treated plants contained only a trace of reserve carbohydrates in the form of starch and dextrin. Depletion of starch and dextrin reserves in most parts of the plants was clearly evident

TABLE 3

TOTAL SUGAR AND STARCH-DEXTRIN CONTENTS OF MORNING-GLORY PLANTS GROWN IN GREENHOUSE UNDER CONDITIONS FAVORABLE FOR VIGOROUS VEGETATIVE GROWTH AND SPRAYED WITH AQUEOUS MIXTURE CONTAINING 1000 P.P.M. 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. FIGURES REPRESENT MILLIGRAMS OF CARBOHYDRATES IN THIRTY PLANTS

| Days | Roots | | Hypocotyls | | Stems | | Buds | | Leaves | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C* | T | C | T | C | T | C | T | C | T | C | T |
| | Sugar | | | | | | | | | | | |
| 0 | 324 | ..... | 52 | ..... | 431 | ..... | 36 | ..... | 260 | ..... | 1103 | ..... |
| ¾ | 246 | 183 | 39 | 33 | 475 | 537 | 54 | 29 | 352 | 374 | 1166 | 1156 |
| 1¾ | 267 | 324 | 39 | 44 | 475 | 572 | 84 | 30 | 280 | 648 | 1145 | 1618 |
| 3 | 304 | 266 | 40 | 58 | 412 | 532 | 77 | 17 | 295 | 681 | 1128 | 1554 |
| 4 | 341 | 225 | 55 | 64 | 487 | 519 | 88 | 20 | 243 | 766 | 1214 | 1594 |
| 6 | 462 | 192 | 53 | 78 | 390 | 675 | 51 | 25 | 148 | 1222 | 1104 | 2192 |
| 8 | 247 | 107 | 48 | 74 | 389 | 561 | 164 | 18 | 225 | 789 | 1073 | 1549 |
| 11 | 453 | 59 | 60 | 52 | 440 | 274 | 222 | 4 | 190 | 613 | 1365 | 1002 |
| 13 | 389 | 28 | 113 | 32 | 280 | 175 | 159 | 1 | 120 | 430 | 1061 | 666 |
| 16 | 52 | 19 | 52 | 50 | 446 | 224 | 369 | 11 | 238 | 314 | 1157 | 618 |
| 20 | 758 | 38 | 100 | 32 | 758 | 146 | 739 | 10 | 1008 | 310 | 3363 | 536 |
| | Starch-dextrin | | | | | | | | | | | |
| 0 | 400 | ..... | 222 | ..... | 749 | ..... | 37 | ..... | 2917 | ..... | 4325 | ..... |
| ¾ | 429 | 393 | 275 | 242 | 894 | 917 | 40 | 19 | 2645 | 2163 | 4283 | 3734 |
| 1¾ | 570 | 434 | 300 | 258 | 1325 | 1226 | 45 | 12 | 2553 | 2613 | 4793 | 4543 |
| 3 | 774 | 391 | 430 | 267 | 1947 | 875 | 64 | 7 | 2830 | 1887 | 6045 | 3427 |
| 4 | 807 | 437 | 401 | 253 | 2375 | 704 | 68 | 1 | 2970 | 1354 | 6621 | 2749 |
| 6 | 1072 | 240 | 635 | 237 | 4030 | 897 | 109 | 10 | 4494 | 936 | 10340 | 2320 |
| 8 | 1093 | 182 | 640 | 168 | 4279 | 486 | 172 | 1 | 2492 | 436 | 8676 | 1273 |
| 11 | 1475 | 161 | 655 | 116 | 5057 | 435 | 396 | 4 | 1536 | 259 | 9119 | 975 |
| 13 | 1743 | 120 | 443 | 81 | 5328 | 210 | 489 | 5 | 1618 | 81 | 9621 | 497 |
| 16 | 1698 | 73 | 640 | 70 | 5711 | 116 | 723 | 1 | 2357 | 11 | 11129 | 271 |
| 20 | 2882 | 21 | 633 | 6 | 6478 | 0 | 1393 | 0 | 3108 | 0 | 14494 | 27 |

* C, control; T, treated.

The starch and dextrin content of treated plants decreased steadily from the fourth day following treatment, and on the twentieth day the sprayed plants contained approximately one five-hundredth as much starch and dextrin by weight as did the untreated ones.

Four days following treatment the

on the third day following treatment (table 3).

During the third week after treatment a marked increase in the weight of the untreated plants occurred which was associated with a corresponding increase in their carbohydrate content. This flush of growth on the part of unsprayed plants

was probably due to increased soil fertility resulting from the application of manure during the first week following treatment.

## Discussion

Responses of morning-glory plants which were vigorously growing and sprayed with 2,4-dichlorophenoxyacetic acid were similar to those of other plants that were in a relatively dormant condition when treated with the acid. Treatment inhibited both vegetative growth and seed development, and when measured in terms of length of time required for death to occur, the effectiveness of this acid was approximately the same on both the actively growing and the relatively dormant plants.

The sugar content of sprayed plants first increased above that of untreated plants, then decreased, while the starch-dextrin reserves were rapidly depleted as the result of treatment. The readily available carbohydrate reserves, especially those in the roots and flower buds, were rapidly depleted following treatment (figs. 3, 4). Depletion of the carbohydrates was apparently a factor which resulted in death of the plants. It has been reported (**5, 6**) that starch and dextrin were depleted in bean leaves treated with naphthaleneacetic acid or certain other growth-regulating substances, and that sugar accumulated more slowly in illuminated leaves that had been depleted of reserve carbohydrates and then treated than it did in comparable unsprayed ones. In these earlier experiments photosynthesis was apparently inhibited by the application of naphthaleneacetic acid. It is possible that the synthesis of carbohydrates by the morning-glory plants of the present experiment was likewise impaired by treatment with the 2,4-dichlorophenoxyacetic acid.

In sprayed plants, necrotic tissues first appeared in the flower buds. This response was also associated with a marked decrease in the carbohydrate content of

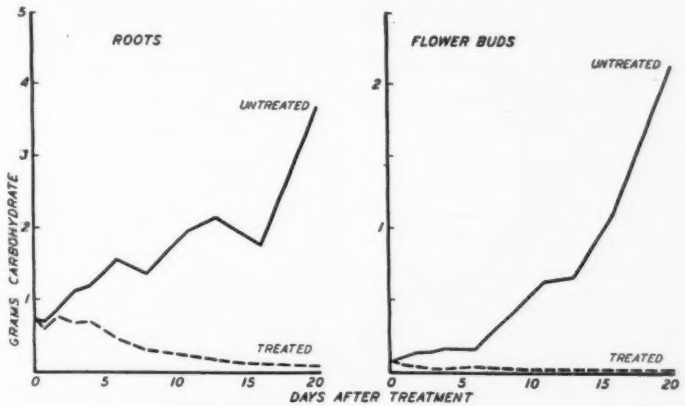


FIG. 4.—Readily available carbohydrate content (sugar, starch, and dextrin) in roots and buds of morning-glory plants during 3-week period following treatment with aqueous mixture containing 1000 p.p.m. 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500, compared with that of untreated plants. Plants grown under conditions favoring vigorous growth. Thirty treated and thirty untreated plants sampled at each period.

the buds and indicated that the reproductive parts of the plants were especially sensitive to the acid treatment. Stems of the treated plants were attacked by soil organisms in the region near the surface of the soil, and the necrotic areas that developed as a result may have also been associated with death of the plants.

The marked changes observed in the relative amounts of the carbohydrate content of the plants treated with 2,4-dichlorophenoxyacetic acid suggested the possibility of utilizing this type of response in controlling the rate of hydrolysis of polysaccharides in other kinds of plants, in their seeds and fruit.

## Summary

1. Plants of annual morning-glory, *Ipomoea lacunosa* L., grown in a greenhouse under conditions favoring vigorous vegetative growth and seed production, were sprayed with an aqueous mixture containing 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500. Other plants of morning-glory grown under conditions of photoperiod and soil fertility less favorable for vegetative growth were treated in a similar way.

2. No apparent growth occurred, either in those treated plants that were in a vigorous vegetative condition or in those that were relatively dormant at the time of treatment. Following treatment, the total dry weight of untreated plants increased while that of the sprayed ones decreased. Neither those that were vegetative nor the dormant ones showed external evidence of gall formation or root initiation as the result of treatment. In the sprayed plants, necrosis was first evident in the flower buds, and none of these developed mature seeds. Both the vegetative and the nonvegetative plants died within 3 weeks following treatment.

3. Readily available carbohydrates (sugars, starch, and dextrin) were essentially depleted within a period of 3 weeks in plants that were growing vigorously, and also in plants that were relatively dormant when treated. Carbohydrate reserves (starch and dextrin) were rapidly depleted in the flower buds, and also in the roots, of the sprayed plants, a response of significance in connection with the use of 2,4-dichlorophenoxyacetic acid in the control of weeds.

4. Sugars in treated plants at first increased above the amount in the untreated ones, then decreased, and they were nearly depleted during the second and third week after treatment.

The collecting, sectioning, and drying of the plant material was done by CHARLOTTE F. GAETJENS and JOHN M. JONES; LAURA E. RAPPLEYE assisted with microchemical tests; and JOHN N. YEATMAN assisted with the preparation and chemical analysis of the samples.

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# HERBICIDAL PROPERTIES OF 2,4-DICHLOROPHENOXYACETIC ACID APPLIED IN DUSTS CONTAINING HYGROSCOPIC AGENTS

PAUL C. MARTH,[1] F. F. DAVIS,[2] AND JOHN W. MITCHELL[3]

## Introduction

Interest in the dust method of applying growth-regulating substances to plants has been manifest because of the rapidity, uniformity, and relatively low costs of application. In some instances the results obtained have compared favorably with those secured through the use of comparable amounts applied in aqueous solutions (1, 2).

In the present experiments, 2,4-dichlorophenoxyacetic acid was applied to various species of weeds grown under greenhouse conditions to determine the effectiveness of the acid as a selective herbicide (*a*) when used with various kinds of dust carriers, and (*b*) when applied with a mixture of carrier and certain water-absorbing agents.

## Preparation of dust mixtures

Finely ground Pyrax, fuller's earth, "Cherokee Clay," and purified talc were tested as carriers. To prepare the various dust mixtures, the desired amount of 2,4-dichlorophenoxyacetic acid was first dissolved in 95% grain alcohol of sufficient volume to produce a thin paste when stirred into a weighed amount of the carrier (approximately 1 ml. of alcoholic solution per gram of dust). After stirring, the paste mixture was placed in a well-ventilated oven at 80° C. and the alcohol evaporated. When dry, the preparation was thoroughly repulverized while warm and then either stored in air-tight containers or used immediately. In preparing dust mixtures with such hygroscopic agents as Carbowax 1500 or glycerin, the same procedure was followed except that the growth-regulating substance was first dissolved in the required amount of hygroscopic agent and then this solution was added to the alcohol.

In preliminary experiments it was found that 3.0% (by weight) of either Carbowax 1500 or glycerin in the carrier produced a dust that was slightly sticky, but one that could be readily applied. The use of higher concentration of the hygroscopic agents resulted in dusts that were very sticky and difficult to apply.

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Calcium chloride used as the hygroscopic agent was finely ground and mixed with the dust in the dry state after the required amount of the acid had been added as just described.

The final dust mixtures were applied in weighed amounts at the rate of approximately 4 pounds per 1000 square feet, or 1.8 gm. per square foot, by means of a small mechanical duster operating at 9 pounds of air pressure.

Both the grass and the clover germinated and grew uniformly, so that the soil surface of the flats was completely covered by the mixture of the two species by February 21, when they were treated. Dust mixtures containing 0.5, 1.0, 2.5, 5.0, and 10.0%, respectively, of 2,4-dichlorophenoxyacetic acid were prepared with each of the carriers—Pyrax, fuller's earth, and Cherokee Clay.

Each of the forty-eight flats was divided crosswise into three plots of equal size, and each of the forty-five dust mixtures was applied to three plots at random, leaving three randomized plots as untreated controls. Immediately prior to dusting, all plots were exposed to a fine aqueous mist, so that moisture was deposited on the foliage to simulate a heavy dew. Records of the effect of treatment on the grass and clover were obtained at the end of 2, 15, and 50 days following treatment.

The data on the Pyrax dust plus the acid treatments only are presented in table 1, since the results with fuller's

TABLE 1

EFFECTS OF VARYING CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID APPLIED IN DUST FORM TO KENTUCKY BLUEGRASS AND WHITE CLOVER GROWING TOGETHER IN GREENHOUSE FLATS. DUST PREPARATIONS MADE WITH PYRAX AND APPLIED FEBRUARY 21, 1945

| Concentration of acid (%) | Extent of injury following treatment | | | | | |
|---|---|---|---|---|---|---|
| | 2 days | | 15 days | | 50 days | |
| | Grass | Clover | Grass | Clover | Grass | Clover |
| 0.5 | o* | + | o | + | o | o |
| 1.0 | o | + | o | + | o | o |
| 2.5 | o | + | + | +++ | o | ++++ |
| 5.0 | + | ++ | ++ | ++++ | + | ++++ |
| 10.0 | +++ | +++ | +++ | ++++ | ++ | ++++ |

* o = No apparent injury.
\+ = Slight injury to grass or curling of clover.
++ = Moderate injury to grass or curling of clover.
+++ = Severe injury to grass or curling of clover.
++++ = Killing of clover (85–100% dead).

## Investigation

### Pyrax, fuller's earth, and Cherokee Clay as carriers

Kentucky bluegrass, white clover.—On January 6, 1945, each of forty-eight flats (12 × 26 inches) containing fertile potting soil was sown uniformly with 2.83 gm. Kentucky bluegrass and 0.70 gm. white clover, rates approximately equivalent to 3 lb. and $\frac{3}{4}$ lb. of seed per 1000 square feet, respectively. After seeding, the flats were maintained in a greenhouse at approximately 60° F.

earth and Cherokee Clay were of the same order. Only the relatively high concentrations of the acid (2.5–10%) effectively controlled the clover. Both the 0.5 and the 1.0% concentrations were ineffective, and at the end of 50 days the clover had recovered completely from the initial curling effects that were apparent at the end of 2 days. Dust applications containing 2.5% concentration of the acid were without serious effect on the bluegrass but greatly reduced the growth of the clover. An estimated 15% of clover remained in these plots at the end of 50 days following treatment. The use of dust with 5.0% of the acid resulted in a moderate amount of tip-burn on the bluegrass foliage but apparently did not greatly retard its development. A dense stand of grass developed in these plots, while the clover was almost completely killed (99%). The bluegrass developed considerable foliage injury when treated with dusts containing 10.0% of the acid. In these treatments the grass had not recovered at the end of 50 days, and only a thin stand was growing at that time (fig. 1). In contrast, all the white clover was killed by the 10.0% acid-dust treatments. No marked differences in behavior of bluegrass or clover were observed in comparing the three carriers at the various concentrations of acid used. From this standpoint it would appear that each of these materials would be satisfactory as a carrier for this acid.

HEAL-ALL, HYDROCOTYLE, AND PLANTAIN.—A 10.0% concentration of 2,4-dichlorophenoxyacetic acid in Pyrax was applied to pure stands of heal-all (*Prunella vulgaris*) and hydrocotyle (*Hydrocotyle rotundifolia*) growing in flats, and also to narrow-leaved plantain (*Plantago lanceolata*) established in potting soil contained in 3-inch clay pots. These plants were maintained in the same greenhouse section at 60° F. and treated on February 21 in a manner and rate similar to that described for the bluegrass-clover experiment. At the time of treatment the foliage of the heal-all and hydrocotyle covered most of the soil surface of each flat. Three areas, consisting of one third of the flat each, were treated in the case of heal-all and hydrocotyle, leaving three untreated areas of equal size. Fifteen each of treated and untreated plantain plants were used.

The 10.0% acid dust proved to be toxic to all three species. The hydrocotyle and heal-all were severly curled within 48 hours after treatment. After 15 days all dust-treated plants of these two species were dead, while the narrow-leaved plantains were severely curled and chlorotic at this time and were dead a month later.

### ADDITION OF WATER-ABSORBING AGENTS TO ACID-TALC DUSTS

PRELIMINARY TESTS.—Several hygroscopic substances were tested for their relative merits as moisture-absorbing agents. A weighed amount of each compound was placed for a period of 164 hours in a closed vessel and exposed to air in which the relative humidity was maintained at approximately 79.5% by means of a saturated solution of ammonium chloride. The percentage increase in weight was 130.9 for calcium chloride (anhydrous), 43.9 for copper sulphate, 40.5 for glycerin, 22.3 for Drierite, 28.2 for Carbowax 1500, 25.0 for Carbowax 400, 17.9 for Carbowax 1540, and 11.1 for pumpkin-fruit tissue (dried and powdered). Of these substances, calcium chloride, Carbowax 1500, and glycerin were selected for use in the dust experiments. The use of polyethylene glycols (Carbowax) in applying growth substances to plants has recently been described (3).

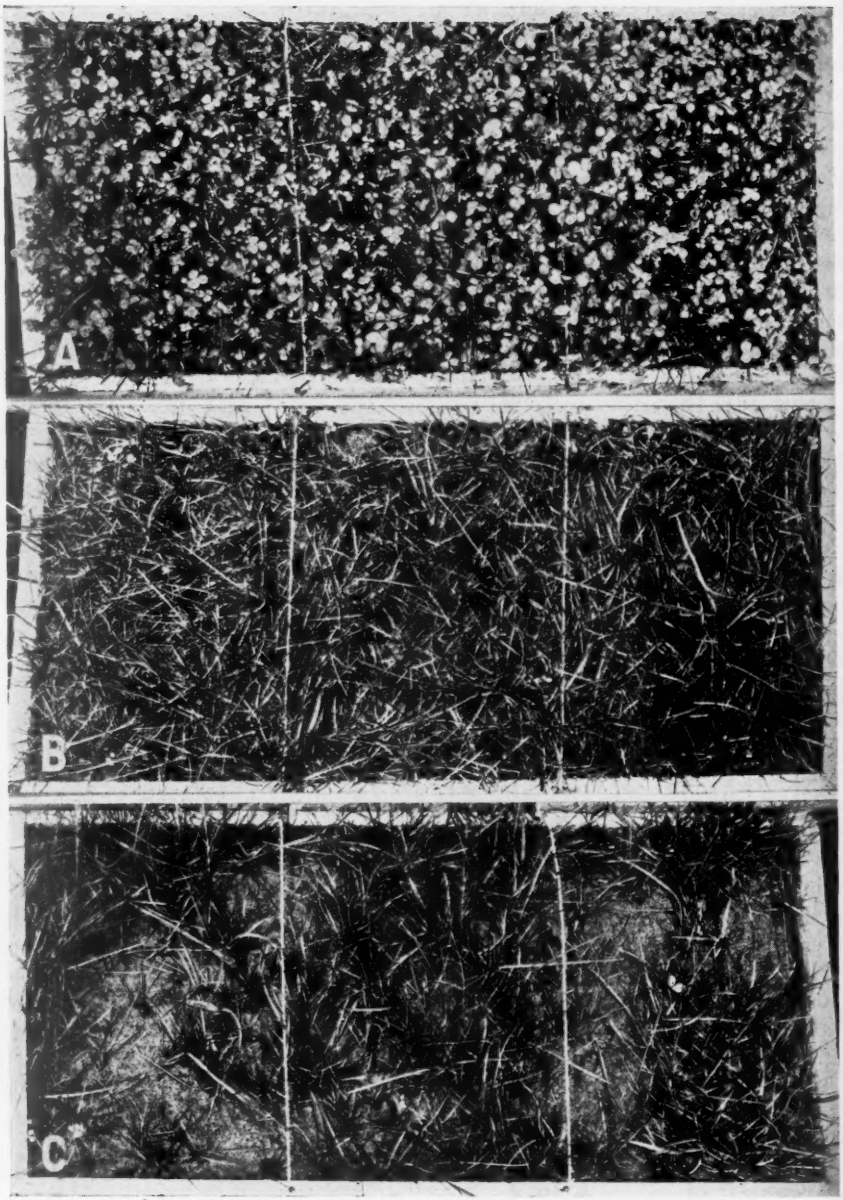


FIG. 1.—Effect of dusts containing 2,4-dichlorophenoxyacetic acid on eradication of white clover from Kentucky bluegrass growing in the greenhouse: *A*, control plots; *B*, plots treated with 5.0% of the acid applied in three dust carriers (left, Pyrax; middle, fuller's earth; right, Cherokee Clay); *C*, plots treated with dusts containing 10.0% of the acid (left, Pyrax; middle, Cherokee Clay; right, fuller's earth). Note excellent control of clover in *B* and *C* as well as poor stand of bluegrass in *C*. Treated February 21; photographed April 13, 1945.

MORNING-GLORY.—Plants of *Ipomea lacunosa* were grown in a greenhouse from seed sown on January 25, in potting soil contained in 3-inch clay pots. A temperature of 65°–70° F. was maintained. After the seedlings had developed the first pair of true leaves, the plants in 600 pots were thinned to two per pot. In the first experiment (March 7), dust treatments (table 2) were applied to 400 plants of this lot having six leaves, and the same treatments were applied again 1 week later (March 15) to plants having eight leaves. In both experiments, twenty plants (ten pots) per treatment were used. In a third experiment the same treatments were applied on May 28 to another set of morning-glory plants that had developed four to five leaves. These plants were selected from 300 pots sown on April 30. Only plants of marked uniformity of size and appearance were used, and the dust applications were made to the dry foliage at the rate previously stated.

Uniform results were obtained in the three experiments with morning-glory. The effectiveness of the acid applied in dust form was markedly increased by inclusion of a moisture-absorbing agent in the dusts (table 2). This effect was most noticeable at the lower concentrations used—0.01 and 0.05%. At the 0.01% concentration the regular acid dust had no apparent effect on the plants, whereas those plants treated with dusts containing 0.01% of the acid to which 3% of either glycerin or Carbowax 1500 had been added showed marked epinasty and stem bending at the end of 48 hours, and these plants failed to grow an appreciable amount during the 4-week period following treatment. In contrast, the treated plants that received the same amount of the acid, but with calcium chloride added, showed slight bending but recovered and produced approximately the same amount of vegetative growth as did the untreated plants during the 4 weeks following treatment.

Differential effects between the three hygroscopic agents were most noticeable with dusts containing 0.05% of the acid. At this concentration and without hygroscopic agents added the plants recovered from the effects of treatment.

TABLE 2

RESPONSE OF MORNING-GLORY PLANTS TO APPLICATION OF 2,4-DICHLOROPHENOXYACETIC ACID IN TALC-DUST MIXTURES CONTAINING MOISTURE ABSORBING AGENTS. RESULTS OF THREE GREENHOUSE EXPERIMENTS (MARCH 7, MARCH 15, AND APRIL 30, 1945) COMBINED

| CONCENTRATION OF ACID (%) | NO HYGROSCOPIC AGENT ADDED TO DUST | | CARBOWAX 1500 (3%) ADDED TO DUST | | GLYCERIN (3%) ADDED TO DUST | | CALCIUM CHLORIDE (3%) ADDED TO DUST | |
|---|---|---|---|---|---|---|---|---|
| | 48 hours | 4 weeks | 48 hours | 4 weeks | 48 hours | 4 weeks | 48 hours | 4 weeks |
| 0..... | o* | o | o | o | o | o | o | o |
| 0.01..... | o | o | + | ++ | + | ++ | + | o |
| 0.05..... | + | o | ++ | ++++ | + | +++ | + | + |
| 0.1..... | + | + | +++ | ++++ | ++ | +++ | + | ++ |
| 0.5..... | ++ | ++++ | +++ | ++++ | +++ | ++++ | ++ | ++++ |

* o = No apparent effect.
+ = Slight bending but plants continued linear growth.
++ = Moderate bending with sharp checking of linear growth.
+++ = Severe bending with complete stoppage of linear growth.
++++ = All plants dead.

Addition of 3% Carbowax 1500 to the dust resulted in the death of all the treated plants after 4 weeks, while plants that received the same amount of the acid but with glycerin added were injured severely and apparently unable to recover, although some were still alive at the end of this period.

Calcium chloride added to the dusts did not inhibit growth as much as did either the Carbowax or glycerin mixtures, even though calcium chloride itself was by far the most effective moisture-absorbing agent of the three compounds tested. The ineffectiveness of the chloride dust may possibly be due to differences in its physical properties in relation to cohesion to the plants, or possibly to chemical reactions with the acid.

In these experiments with morning-glory, greatest differences in herbicidal effects of the several dust mixtures were noted at 0.5% concentration. The greatest initial response at this concentration was found in plants treated with dusts containing either Carbowax or glycerin. All plants treated with any dust mixture containing 0.5% acid died within 4 weeks following treatment, and at this concentration and lapse of time the effect of adding a water-absorbing agent was no longer apparent.

RAGWEED.—Plants of *Ambrosia bidentata* growing in 3-inch pots in fertile soil were treated with the same dust mixtures and at the same rates as described for the morning-glory tests of March 15. When dusted, the plants were 4–5 inches in height and were growing vigorously. Ten plants were included in each of the treatments.

Ragweed failed to show a noticeable effect when treated with dust containing 0.1% or less of the acid, while at a 0.5% concentration inhibition of growth occurred only in the case of those dusts containing 3% of either Carbowax 1500 or glycerin. The plants in these last-mentioned lots, however, were not killed by treatment at the end of 4 weeks, although they had made no apparent growth during this period.

LAMB'S-QUARTERS.—Ten plants each of *Chenopodium album* growing in 3-inch pots were included with the April 30 dusting treatments on morning-glory. The plants were 6–7 inches in height and growing vigorously when treated.

Inclusion of a moisture-absorbing agent increased the effect of the acid on the plants (fig. 2). Dusts containing 0.1% or less of concentration had no apparent effect, except in the case of a few plants which later recovered completely. The plants of lamb's-quarter were killed by applications of dust containing 0.5% of the acid and 3% of either Carbowax 1500 or glycerin.

## Discussion

It is evident that 2,4-dichlorophenoxyacetic acid is relatively active when applied in dust carriers, especially when a water-absorbing agent is added to the mixture. On the basis of the present experiments, somewhat larger amounts of the acid were required to bring about a given response when applied in dust carriers than were required for equal effects with liquid sprays (4).

A factor which may restrict to some extent the use of growth regulators in dust carriers is the difficulty of confining the dust treatments within the necessary area. This difficulty may be particularly serious in connection with the use of the acid as a herbicide. In eradicating weeds, the accidental treatment of sensitive crop plants would be more likely to occur during the application of dust treatments than during the use of sprays.

Treatment of large areas of turf, grass-

lands, or cultivated grass crops with dusts for the purpose of selective eradication of certain weeds appears to be feasible, but further experimental work is needed to determine the usefulness of this method of application under a variety of field conditions. The results reported here with the various acid-dust mixtures were with application to plants in a greenhouse, and no attempt was made to simulate intermittent dews, rains, and other weathering conditions commonly found in the field. These conditions might modify results with the various combinations used in the work reported here.

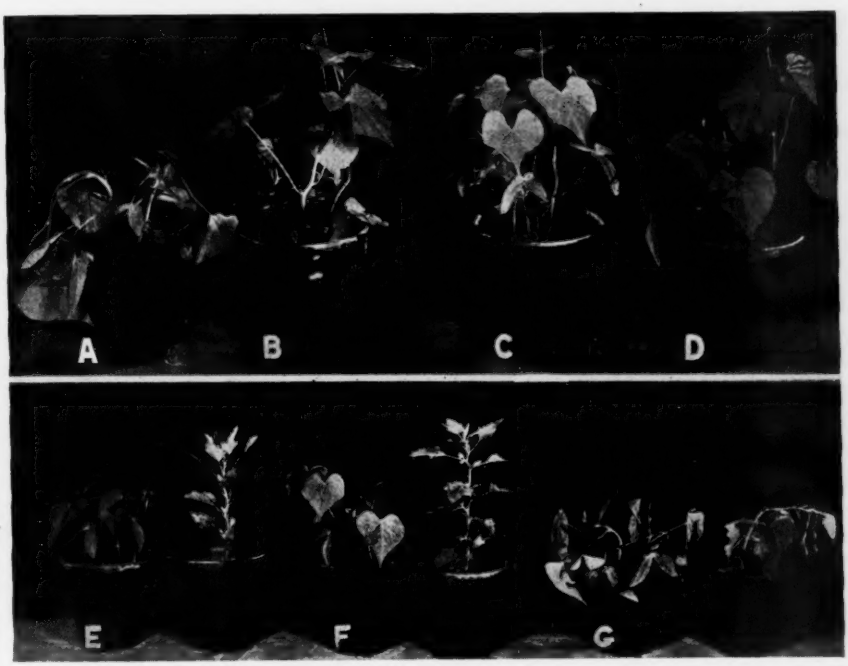


FIG. 2.—Effect of addition of hygroscopic agents to talc-dust mixtures containing 2,4-dichlorophenoxyacetic acid: *A*, morning-glory (*Ipomea lacunosa*) plants treated with talc containing 0.1% of acid plus 3.0% of Carbowax 1500 as hygroscopic agent. *B*, similar plants treated with dust containing same amount of acid but without hygroscopic agent. *C*, plants treated with talc dust containing 3.0% of Carbowax only. *D*, untreated control. *E*, morning-glory at left, lamb's-quarters (*Chenopodium album*) at right, untreated control plants. *F*, similar plants treated with talc dust containing 0.5% acid only. *G*, comparable plants that received dust containing 0.5% acid plus 3.0% glycerin as hygroscopic agent. Photographs taken 3 days after treatment (April 30, 1945). Plants in treatments *A* and *G* were dead 28 days following treatment, while plants in all other treatments were apparently unaffected.

## Summary

1. 2,4-Dichlorophenoxyacetic acid was applied in different concentrations (0.5, 1.0, 2.5, 5.0, and 10.0%) in dust carriers, at the rate of 4 pounds per 1000 square feet, to Kentucky bluegrass (*Poa pratensis*) and white clover (*Trifolium repens*) growing in a mixed stand in greenhouse flats. The 10.0% dusts were also tested

on heal-all (*Prunella vulgaris*) and lawn pennywort (*Hydrocotyle rotundifolia*) in flats and on narrow-leaved plantain (*Plantago lanceolata*) growing in 3-inch clay pots in a greenhouse.

2. Comparison of Pyrax, fuller's earth, and Cherokee Clay as diluents or dust carriers at each of the concentrations indicated that there was no marked difference among them on the basis of the plant responses observed.

3. Complete eradication of clover from bluegrass turf was obtained by applications of dust containing 10.0% of the acid, but the bluegrass was severely injured and recovered only slowly. Almost complete eradication (99%) was obtained by application of dust containing 5.0% of the acid, and the grass recovered quickly from the slight injury that developed. A minimum concentration of 2.5% of the acid was necessary to kill an appreciable amount (85%) of clover. No bluegrass injury was observed at this concentration.

4. Heal-all, lawn pennywort, and plantain were likewise killed by application of dusts containing 10% of the acid.

5. Addition of hygroscopic agents, such as Carbowax 1500 or glycerin, at the rate of 3% markedly increased the effects of the growth-regulating substance in dust mixtures. Addition of calcium chloride to the regular dusts resulted in only a slight increase in effectiveness of the acid. Plants of morningglory (*Ipomea lacunosa*) were killed by dusts containing 0.05% concentration of acid plus 3% Carbowax 1500, while comparable plants were but slightly affected and recovered from treatment with the same kind of dust having an equal concentration but no hygroscopic agent added.

6. Plants of ragweed (*Ambrosia bidentata*) were not evidently affected by the application of dusts containing 0.5% of the acid. Comparable plants exhibited severe epinasty and failed to grow following treatment with a 0.5% acid dust to which either Carbowax 1500 or glycerin had been added.

7. Similar results were obtained with plants of lamb's-quarters (*Chenopodium album*), which were killed after 4 weeks by application of dust containing 0.5% of acid and 3% of Carbowax 1500 or dust containing the same amount of the acid but with glycerin added. The plants were but slightly affected by comparable dusts that did not include the hygroscopic agents.

8. Caution should be exercised in applying 2,4-dichlorophenoxyacetic acid by the dust method, first to avoid accidental treatment of adjacent plants by drifting of the dust particles, and second to avoid breathing the dust mixtures during application until definite information is available on the nontoxicity of the acid.

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# CURRENT LITERATURE

*Trees and Toadstools.* By M. C. RAYNER. London: Faber & Faber, Ltd., 1945. Pp. 71. Illustrated. 6*s*.

This is a short but lucid account of the interrelationships between certain of the higher fungi and trees. Although primarily designed for the general reader, the book will nevertheless redirect the attention of many botanists to the curious morphological and nutritional features of the associations between higher fungi and trees which the author is so well qualified to describe. "Fungus roots" and the effects of the mycorhiza-forming fungus on the growth of conifers are described and illustrated by photographs.—F. C. STEWARD.

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*The Structure and Reproduction of the Algae*, Vol. II. By F. E. FRITSCH. Cambridge, University Press; New York, Macmillan Co., 1945. Pp. xii+939. $12.00.

The second of a two-volume treatise on the structure and reproduction of the algae has now been published, following the first volume by approximately 10 years. The treatise has been designed to present in the English language a comprehensive account of the morphology of the algae, a task for which the author, who has devoted a fruitful life to this group of plants, is particularly qualified.

FRITSCH has arranged the algae into eleven classes, as follows: Chlorophyceae (Isokontae), Xanthophyceae (Heterokontae), Chrysophyceae, Bacillariophyceae (diatoms), Cryptophyceae, Dinophyceae (Peridinieae), Chloromonadineae, Euglinieae, Phaeophyceae, Rhodophyceae, and Myxophyceae (Cyanophyceae). The first eight of these classes were included in volume I, leaving the last three for volume II.

Throughout both volumes the treatment is essentially morphological, with taxonomic and biologic problems discussed only where they are thought to have a bearing on morphology. The literature of the world has been surveyed and the pertinent facts bearing on the morphology of algae have been carefully and critically assayed and incorporated in both volumes. Essentially all the literature which has either practical or historical value has been included at the end of each section dealing with an order. The citations cover the first half of 1943.

These two volumes should form an essential part of the biological library of every college and university. They are likely to prove equally necessary to all who have a special interest in the algae.—J. M. BEAL.

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*A Catalog of Illinois Algae.* By MAX E. BRITTON. Evanston, Ill.: "Northwestern University Studies in the Biological Sciences," No. 2, 1945. Pp. viii+177. $3.00.

This catalog of the Illinois algae brings together all the algae which have been reported in slightly more than ninety previously published papers. It lists a total of 178 genera and 962 species, varieties, and forms distributed among the following classes: Cyanophyta 29 genera and 98 species, varieties, or forms; Rhodophyta 2–2; Chrysophyta 51–308; Chlorophyta 88–509; Pyrrophyta 3–11; and Euglenophyta 5–34. In a short chapter the literature is reviewed under certain categories—(*a*) taxonomic and floristic studies, (*b*) general ecological and pollination studies, (*c*) studies of algae as animal food supply, (*d*) monographs and other comprehensive reports, and (*e*) miscellaneous papers. For each species, variety, and form listed, the place and date of collection, with the name of the collector, are given. This book should prove highly useful to all those interested in the algae of the midwest region of the United States.—J. M. BEAL.